



European guidelines for quality assurance in cervical cancer screening

Second Edition

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Prefaces

Preface

Markos Kyprianou*

Cytological screening every three to five years can prevent up to four out of five cases of cervical cancer. Such benefits can only be achieved if screening is provided in organized, population-based programmes with quality assurance at all levels. This is an important lesson which has been learned through pan-European cooperation and collaboration in the European Cancer Network.

The completion of the second edition of the European Guidelines for Quality Assurance in Cervical Cancer Screening is testimony to the unique role the European Union can play in assuring the efficient delivery of safe and effective services to maintain and improve the health of Europe's citizens. Experts from most of the EU member states have collaborated to prepare the updated recommendations and standards for designing, implementing, and monitoring the performance of cervical cancer screening programmes including first guidelines for diagnosis and management of screen detected cervical lesions.

Quality assurance of the screening process requires a robust system of programme management and coordination, assuring that all aspects of the service are performing adequately. The first edition of the European Guidelines for Quality Assurance in Cervical Cancer Screening emphasized the principles of organised, population-based screening and was instrumental in initiating pilot projects in Europe. More than a decade has passed since publication of the first guideline edition.

Subsequently, the Council adopted in December 2003 the Council recommendation on cancer screening recommending to the Member States, whenever available to follow evidence-based EU guidelines for cancer screening in implementing or improving, e.g., national population-based cervical cancer screening programmes. Therefore the appearance of this second comprehensive edition of the EU guidelines for Quality Assurance of Cervical Cancer Screening documents the commitment of the Commission to deliver on the invitation to the Commission by the Council for continued support for the development and dissemination of high quality EU screening guidelines.

The editors and contributors to the current, expanded guideline edition are to be applauded for providing extensive updates on technical aspects and documentation, as well as assessment of new technologies. The current recommendations include uniform indicators for monitoring programme performance and for identifying and reacting to potential problems at an early time. They are particularly relevant to planning new cervical cancer screening programmes in Europe.

This Publication of the second edition of the guidelines by the European Union will ensure that any interested organisation, programme or authority in the Member States as well as every European Citizen can obtain the recommended standards and procedures and appoint appropriate persons, organisations and institutions for the implementation of those.

Let me finally thank the editors and contributors for their efforts in compiling this volume which I am confident will be useful to guide work on cervical cancer screening for the years to come.

Brussels, November 2007

*European Commissioner for Health and Consumer Protection

Preface

Peter Boyle*

Screening for cytological abnormalities and treatment of precursor lesions has contributed significantly to the substantial decline in cervical cancer incidence and mortality rates in Europe over recent decades. Improvements in the control of cervical cancer have been particularly discernible in those countries which have implemented population-based screening programmes with high acceptance of personal invitation. Despite these successes there is no room for complacency in the ongoing effort for cervical cancer control in Europe. Currently ca. 34,000 new cases and over 16,000 deaths due to cervical cancer are reported annually in the European Union. The burden of cervical cancer is particularly high in the newer EU Member States, and reaches levels approximately 10-fold greater than the lowest mortality observed elsewhere in the EU. This disparity could be substantially reduced by implementation of population-based cervical cancer screening programmes, with effective quality assurance throughout the screening process.

The International Agency for Research on Cancer (IARC) has provided scientific and technical support for development of the second edition of the European Guidelines for Quality Assurance in Cervical Cancer Screening. Continuously improved quality assurance guidelines based on scientifically sound and applicable screening standards are essential to assuring that population-based programmes of appropriate quality and effectiveness are available to all women who may benefit from cervical cancer screening.

European countries which have not yet launched screening programmes, and those which have already initiated screening are urged to act on the updated and expanded second edition of the EU Guidelines. Organized, population-based screening programmes should be implemented where they are lacking, and the updated recommendations and standards in the EU Guidelines should also be used to improve the quality and effectiveness of already established screening programmes.

The prevalence of oncogenic human papillomavirus (HPV) types in a number of EU Member States underlines the priority of increasing efforts to implement and improve cervical cancer screening programmes. Despite the urgency in dealing with the burden of cervical cancer in Europe, the guideline editors rightly point out the need for planning prior to screening programme implementation in order to maximise effectiveness and to permit evaluation. Furthermore, cancer registration and linkage of screening data with cancer registry data is essential to monitoring the performance and evaluating the impact of screening programmes. Widespread application of the standardised performance indicators recommended in the guidelines will facilitate quality management and will help to recognize programmes and approaches which are more successful. This, in turn, will promote the international exchange of information and experience between programmes which is essential for continuous quality improvement.

It should also be noted that the fundamental principles of quality assurance of cervical cancer screening elucidated in the EU guidelines also apply to settings in which resource limitations require different test procedures, or a significantly lower number of screening tests per woman, such as once-in-a-lifetime screening with visual inspection. Publication of the updated second edition of the EU guidelines is therefore also an important part of the efforts of the Agency to provide scientific support for regions of the world in which the burden of cervical cancer is still substantially higher than in Europe.

PREFACE

The new second edition of the European guidelines appears at a time in which vaccination against oncogenic HPV types has the potential to become a valuable tool which can supplement, but not replace, the important role played by screening in effective cervical cancer control. As pointed out by the guideline editors, vaccination of young girls may lead to substantial reduction in the burden of cervical cancer in future generations of women. For many years, however, most cervical cancer cases and deaths will occur in women who have not been vaccinated. Vaccination is not an alternative to screening for the coming years.

Development of comprehensive European guidelines on cervical cancer prevention which take both primary and secondary prevention into account is an important aim of IARC activities which will also be pursued in the framework of the recently initiated Guideline updating project coordinated by the Agency and supported by the EU Public Health programme.

Lyon, October 2007

*Director, International Agency for Research on Cancer

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Executive Summary

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Cancer is common in older people but cancer of the uterine cervix primarily affects younger women, with the majority of cases appearing between the ages of 35 and 50, when many women are actively involved in their careers or caring for their families. In the European Union (EU) 34 000 new cases and over 16 000 deaths due to cervical cancer are reported annually (Arbyn *et al.*, 2007a & c).

The burden of cervical cancer is particularly high in the new member states. The highest annual world-standardised mortality rates are currently reported in Romania and Lithuania (13.7 and 10.0/100 000, respectively) and the lowest rates in Finland (1.1/100 000). Governmental authorities, parliamentary representatives and advocates should be aware that the substantially higher dimension of this public health problem in the east of the EU requires special attention.

Among all malignant tumours, cervical cancer is the one that can be most effectively controlled by screening. Detection of cytological abnormalities by microscopic examination of Pap smears, and subsequent treatment of women with high-grade cytological abnormalities avoids development of cancer (Miller, 1993).

Cytological screening at the population level every three to five years can reduce cervical cancer incidence up to 80% (IARC, 2005). Such benefits can only be achieved if quality is optimal at every step in the screening process, from information and invitation of the eligible target population, to performance of the screening test and follow-up, and, if necessary, treatment of women with screen-detected abnormalities.

Quality assurance of the screening process requires a robust system of programme management and coordination, assuring that all aspects of the service are performing adequately. Attention must be paid not only to communication and technical aspects but also to qualification of personnel, performance monitoring and audit, as well as evaluation of the impact of screening on the burden of the disease.

Population-based screening policy and organisation conforming to evidence-based standards and procedures provide the overall programmatic framework essential to implementation of quality assurance and are therefore crucial to the success of any cervical cancer screening programme.

Establishment of screening registries and linkage of individual screening data with cancer registry data, taking into account appropriate data protection standards and methods, are essential tools of monitoring and evaluation.

The first edition of the European Guidelines for Quality Assurance in Cervical Cancer Screening (Coleman *et al.*, 1993) established the principles of organised, population-based screening and was pivotal in initiating pilot projects in Europe. A number of countries have in the meantime developed organised, population-based screening approaches, which are illustrated in the second edition. It is hoped that this new guideline edition will have a greater impact on those countries in which opportunistic, rather than organised, population-based screening has been the preferred model in the past. Toward this end, considerable attention has been given to the essential aspects of developing an organised, population-based programme policy that minimises the adverse effects and maximises the benefits of screening.

The current recommendations are also particularly relevant to planning new cervical cancer screening programmes in Europe. Different solutions fulfilling the recommended methodological standards need to be implemented in different countries and regions with diverse levels of resources and general healthcare infrastructure.

More than a decade has passed since publication of the first guideline edition. The current, expanded edition therefore also includes extensive updates on technical details and documentation,

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as well as assessment of new technologies, e.g.: liquid-based cytology, automated interpretation of Pap smears and testing for human papillomaviruses. The scope of the current guideline has also been extended to include comprehensive instructions prepared by a multi-disciplinary team of experts for general practitioners, gynaecologists and cytopathologists. Much more extensive recommendations on follow-up, diagnosis and management of women with positive cervical cytology have been added. This necessitated the incorporation in the second edition of a separate chapter on techniques and quality assurance in histopathology and, for the first time, detailed guidance for clinicians in dealing with abnormal cytology, including management according to the severity of cytological abnormalities and management of histologically confirmed cervical epithelial neoplasia.

A major further addition has been the inclusion of uniform indicators for monitoring programme performance and for identifying and reacting to potential problems at an early time. The indicators deal with screening intensity, test performance, and diagnostic assessment and treatment, and address aspects of the screening process that influence the impact, as well as the human and financial costs of screening. Standard tables have been provided for documenting screening policies, and for tabulating the person-based data used to generate the uniform performance indicators. The availability of these standardised tools will substantially improve data comparability and the exchange of experience and results between screening programmes in Europe. Such exchange, in turn, is essential to effective pan-European collaboration in implementing and continuously improving the quality and effectiveness of cervical cancer screening programmes.

Cervical cytology still is the cornerstone of cervical cancer prevention programmes in Europe, although new perspectives for other screening technologies are developing rapidly. The principles of quality assurance, performance monitoring and evaluation, and many of the procedures and methodological standards laid down in the current guideline edition are of equal relevance to cervical cancer screening based on other conceivable methods. It is therefore expected that the publication of the updated and revised second edition will also promote rigorous standards in the evaluation and application of new screening technologies, thereby improving the effectiveness of cervical cancer prevention in Europe.

Over the short and medium term, screening for cervical cancer precursors and management of screen-detected lesions will remain the most effective tool for cervical cancer prevention in Europe. However, the field of cervical cancer prevention is rapidly developing due to better understanding of the natural history of the disease. Persistent infection with one of 13 to 16 oncogenic human papillomavirus (HPV) types is now known to be a key prerequisite for development of cervical cancer. The overwhelming evidence linking HPV infection to cervical cancer has prompted the development of test systems to detect its nucleic acids as well as prophylactic and therapeutic vaccines.

Primary prevention by prophylactic vaccination against the HPV types that are causally linked with most cervical cancers in Europe, is likely to become a feasible option for cervical cancer control, provided the current cost of inoculation regimens is substantially reduced.

While prophylactic vaccination, primarily in young girls, may provide important future health gains, cervical screening will need to be continued. Neglecting cervical cancer screening due to the current availability of a vaccine could paradoxically lead to an increase in cancer cases and deaths. Development of comprehensive European guidelines on prevention of cervical cancer that appropriately integrate screening and vaccination strategies is a key aim of the next phase of guideline development activities supported by the EU Public Health Programme.

The current updated and expanded second guideline edition has been prepared by a multidisciplinary team of experts appointed by the European Commission from the former European Cervical Cancer Screening Network (ECCSN) established under the Europe Against Cancer Programme. In addition to the cytopathologists, epidemiologists, general practitioners, gynaecologists, histopathol-

ogists, virologists, and specialists in social science serving as editors and authors; experts from outside the ECCSN were also invited to write, review, and contribute to the development of the second edition. Besides the input of the 48 experts from 17 member states directly involved in the production of the guidelines, numerous comments and suggestions were provided by experts attending meetings held in Denmark, Finland, Greece, Hungary and Luxembourg from 2003 to 2006 by the ECCSN and the European Cancer Network (ECN) in which the former cancer screening networks have been consolidated in the current EU Public Health Programme.

A draft revised guideline was made available for public consultation at <http://www.cancer-network.de> in December 2003. The results of this consultation were incorporated into a new draft which was reviewed by experts invited by the International Agency for Research on Cancer (IARC) to Lyon, France, in June 2005. Two or three reviewers were invited for each chapter, in order to comment on the contents and to ensure that all relevant references available had been considered. The further revised guideline content was subsequently discussed with screening experts from 23 member states and one applicant country of the European Union at the ECN network meeting in February 2006. Since then, IARC has provided technical and scientific support to the editorial board and the authors for the final preparation of the guideline document.

The final recommendations and standards of best practice in the revised and updated second guideline edition are based on the expert consensus in the editorial board subsequent to the above-mentioned consultations and discussions. They take into account the available evidence of screening and diagnostic procedures and programmes. For assessing evidence of effectiveness two criteria were used: study type and study outcomes. Study types were ranked from high to low level evidence as following: (1) randomised clinical trials, (2) observational studies: case-control studies, cohort studies and (3) correlational studies (time trends, geographical comparisons). Outcomes of studies were ordered as: (1) reduction of mortality from cervical cancer, (2) reduction of incidence of invasive cervical cancer, (3) reduction of incidence of CIN3 or cancer (CIN3+), (4) increased detection of high-grade histologically confirmed cervical intra-epithelial neoplasia (CIN3+ or CIN2+), (5) increased test positivity rate without or small loss in positive predictive value for CIN2+. Throughout this guideline, scientific evidence on which the recommendations are based is indicated by references in the text. Where no observed data were available, outcomes simulated by mathematical models and expert opinion were accepted as lowest level of evidence.

The authors conducted systematic literature searches and used available systematic reviews and published meta-analyses. Publication of the handbook for cervical cancer prevention by the IARC Working Group on the Evaluation of Cancer Preventive Strategies in 2005, which included several ECN experts, was also helpful. Several pioneering population-based randomised trials have been conducted or are currently being conducted in various member states in recent years: liquid-based cytology (Italy, The Netherlands), automated cytological screening (Finland); HPV-based versus cytology and combined (cytology+HPV) screening (Finland, Italy, Netherlands, Sweden, UK). The results available from these trials were taken into account during the preparation of the second guideline edition up to July 2007. In addition, several meta-analyses were performed to assess the level of evidence of new screening or management methods: liquid-based versus conventional cytology; HPV testing in triage of minor cytological lesions to identify women needing further follow-up, in follow-up after treatment of CIN to predict success or possible failure of treatment; and in primary screening. In the meta-analyses performed for the current guideline edition it was only possible to assess cross-sectional outcomes (outcome types 4-5); an insufficient number of trials had reached longitudinal outcomes prior to final closure of chapter revisions in mid 2007. One additional meta-analysis concerned obstetrical adverse effects of treatment of pre-cancer lesions.

Fundamental points and principles

Screening policy

- The Council of the European Union has recommended implementation of population-based cervical cancer screening programmes to the EU member states, with quality assurance at all levels and in accordance with European guidelines (Council of the European Union, 2003).
- Screening recommended by the European Council and the European Guidelines is set up as a population-based public health programme, with identification and personal invitation of each woman in the eligible target population. In addition to invitation, the other steps in the screening process and the professional and organisational management of the screening service, including quality assurance, monitoring and evaluation, are well defined by programme policy, rules and regulations at the regional and national level.
- Designing a cervical cancer screening programme includes defining the screening policy, i.e. choosing the screening test systems, determining the target age group and the screening interval between normal test results (3 or 5 years), and establishing follow-up and treatment strategies for screen-positive women, taking into account the variation in background risk in target populations and the natural history of the disease, which is characterised by a rather long detectable pre-clinical period and substantial regression rates of the pre-cancerous lesions.
- Cervical cytology is the currently recommended standard test for cervix screening, which should start in the age range 20–30. It is recommended to continue screening at 3-5-year intervals until the age of 60 (Advisory Committee on Cancer Prevention, 2000; Boyle *et al.*, 2003) or 65 (Coleman *et al.* 1993; IARC, 2005). The upper limit should not be lower than 60 years (Advisory Committee on Cancer Prevention, 2000). Stopping screening in older women is probably appropriate among women who have had three or more consecutive previous (recent) normal cytology results.
- Special attention should be paid to the problem of older women who have never attended screening, as they exhibit increased risk for cervical cancer.
- Opportunistic screening, which takes place in clinical settings and depends on the initiative of the individual woman or her doctor, should be discouraged. Such activities are often characterised by high coverage in selected parts of the population which are screened too frequently, coexisting with a low coverage in other population groups with less socioeconomic status, and heterogeneous quality, resulting in limited effectiveness and poor cost-effectiveness.

Screening organisation, monitoring and evaluation

- The programme design must permit evaluation. An experimental design that is suitable for evaluation of new screening policies in organised settings is recommended.
- The success of a screening programme requires adequate communication with women, health professionals and persons responsible for the health care system.
- Moreover, a well-organised screening programme must reach high population acceptance and coverage, and must ensure and demonstrate good quality at all levels.
- The communication strategy for cervical cancer screening must be underpinned by robust ethical principles and ensure that the information developed is evidence-based, 'women-centred' and

delivered effectively, taking into account the needs of disadvantaged groups and enabling women to make an informed choice about participation at each step in the screening process.

- Population-based information must be established for continuous monitoring of screening process indicators. An appropriate legal framework is required for registration of individual data and linkage between population databases, screening files, and cancer and mortality registers. Indicators of screening programme extension and quality need to be regularly published
- The information system is an essential tool for managing the screening programme; computing the indicators of attendance, compliance, quality and impact; and providing feedback to involve health professionals, stakeholders and health authorities.

New screening technologies

- An observation that a new screening method detects more precursor lesions than the standard Pap smear does not sufficiently demonstrate improved effectiveness. Due to frequent regression of precursor lesions, high specificity is also required to avoid anxiety, unnecessary treatment and side effects. Evidence of effectiveness should preferentially be based on reduction of cancer morbidity and mortality. Nevertheless, reduction in incidence of grade 3 cervical intraepithelial neoplasia (CIN3), is a surrogate indicator of effectiveness.
- Prior to routine implementation of a new screening strategy, the feasibility, cost-effectiveness and quality assurance should be verified and the necessary training and monitoring should be organised. A randomised screening policy, which permits quality-controlled piloting of a new test or procedure in the context of an organised screening programme, is a particularly powerful tool for timely evaluation under real-life conditions.

Cytological methods

- The occurrence of false-negative and unsatisfactory Pap smears has prompted the development of liquid-based cytology (LBC) and automated screening devices. The quality of the evaluation of the performance of these technologies often was poor and rarely based on histologically defined outcomes using randomised study designs. In general, the proportion of unsatisfactory samples is lower in LBC compared to conventional cytology, and the interpretation of LBC requires less time. The cost of an individual LBC test is considerably higher, but ancillary molecular testing, such as high-risk HPV testing in the case of ASC-US, can be performed on the same sample. The economic advantage of LBC due to the reduction of recalls for a new sample depends on the existing rates of inadequate Pap smears, which are highly variable throughout Europe.
- An Italian population-based randomised study, recently confirmed that the sensitivity of LBC and conventional cytology are similar.
- Computer-assisted screening using LBC is currently being evaluated, but insufficient evidence is available for guidelines.

HPV-detection

- Several applications for HPV DNA detection have been proposed: 1) primary screening for oncogenic HPV types alone or in combination with cytology; 2) triage of women with equivocal cytological results; 3) follow-up of women treated for CIN to predict success or failure of treatment.
- HPV infections are very common and usually clear spontaneously. Detection of HPV DNA thus carries a risk of unnecessary colposcopies, psychological distress and possibly of overdiagnosis. The need to perform cervical cancer screening in an organised programme, rather than in an opportunistic setting, therefore applies particularly to screening based on HPV testing.

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- Evidence from randomised studies and meta-analyses shows that triage of women with equivocal cytological lesions by HPV testing with the Hybrid-Capture 2 assay is more sensitive and equally specific in finding high-grade CIN compared to repeat cytology. There is also evidence indicating that HPV DNA detection predicts treatment failure more quickly than cytological follow-up.
- The high sensitivity of current HPV DNA detection methods yields very high negative predictive values even for adenocarcinoma precursors that often escape cytological detection. Recent cohort studies indicate a prolonged duration (up to ten years) of the negative predictive value of HPV testing. Nevertheless, further longitudinal research is necessary, preferably in an organised setting guaranteeing optimal follow-up, using randomised designs and targeting relevant outcomes.
- Current randomised controlled trials may demonstrate lower cumulative incidence of CIN3 and invasive cervical cancer as joint or separate outcomes in HPV-negative compared to cytology-negative women. The results of these trials are needed before screening policies for general primary HPV screening can be recommended in Europe. Such policies would also have to ensure that possible increases in the detection and management of less severe lesions are kept to an appropriate minimum. Introduction of primary HPV screening will require appropriate triage and counseling of HPV-positive women.
- Primary HPV screening should not be recommended without specifying the age group to be targeted, the screening interval, and the essential elements of quality assurance required for programme implementation. HPV screening in an opportunistic setting is not recommended, because adherence to the appropriate intervals and requisite quality control cannot be adequately assured under such conditions.
- Piloting with validated HPV DNA testing can be recommended if performed in an organised screening programme with careful monitoring of the quality and systematic evaluation of the aimed outcomes, adverse effects and costs. Rollout towards national implementation can be considered only after the pilot project has demonstrated successful results with respect to effectiveness (relative sensitivity, positive predictive value of the screening test, triage and diagnostic assessment) and cost-effectiveness, and after key organizational problems have been adequately resolved.

Guidelines for cytology laboratories

- Professional and technical guidelines must be followed to assure the collection and preparation of an adequate cervical cell sample (Arbyn *et al.*, 2007b).
- The quality of a cervical cytology laboratory depends on adequate handling and staining of the samples, screening and interpretation of the slides and reporting of the results. An appropriate balance must be achieved between the best patient care possible, laboratory quality assurance and cost effectiveness (Wiener *et al.*, 2007).
- Uniform grading of cellular abnormalities is an essential condition for registration and comparisons over time and between different settings. Laboratories should apply only a nationally agreed terminology for cytology that is translatable into the Bethesda reporting System (Herbert *et al.*, 2007). The CIN terminology should be reserved for describing histology.

Guidelines for histopathology

- Histopathology provides the final diagnosis on the basis of which treatment is planned, and serves as the gold standard for quality control of cytology and colposcopy. It is also the source of the diagnostic data stored at the cancer registry and used for evaluation of screening pro-

grammes. It is therefore important that histopathology standards are monitored and based on CIN or other internationally agreed-upon terminology.

- Histopathologists should be aware of, and familiar with, the nature of cytological changes that may be relevant to their reports.
- The accuracy of the histopathological diagnosis of tissue specimens depends on adequate samples, obtained by colposcopically directed punch biopsies (with endo-cervical curettage if necessary) or excision of the transformation zone or conisation. An accurate histological diagnosis further depends on appropriate macroscopic description, technical processing, microscopic interpretation and quality management correlating cytological and histological diagnosis.

Guidelines for management of screen-positive women

- A woman with a high-grade cytological lesion, a repeated low-grade lesion or with an equivocal cytology result and a positive HPV test should be referred for colposcopy. The role of colposcopy is to identify the location of the abnormal cells, to target taking of biopsies and to decide whether any treatment is required. Colposcopy should only be performed by adequately trained health professionals.
- Colposcopy is sometimes proposed as an alternative screening method, but its specificity (and probably also its sensitivity) in primary screening is too low for this purpose.
- Guidelines are provided for the management of atypical squamous cells of undetermined significance (ASC-US) and high-grade squamous intra-epithelial lesions (HSIL). Guidelines for low-grade squamous intraepithelial lesions (LSIL) are difficult to delineate because current evidence does not indicate that any method of management is optimal. Repeat cytology or colposcopy are acceptable options, but HPV testing as an initial management option is not sufficiently selective for all women with LSIL. However, HPV testing in older women with LSIL can be considered.
- Quality assurance and collection of data on patient management are important elements of the management and follow-up of women referred with an abnormal cervical smear.

References

Advisory Committee on Cancer Prevention (2000). Recommendations on cancer screening in the European Union. *Eur J Cancer* **36**, 1473-1478.

Arbyn M., Autier P., & Ferlay J. (2007a). Burden of cervical cancer in the 27 member states of the European Union: estimates for 2004. *Ann. Oncol.* **18**, 1425-7.

Arbyn M., Herbert A., Schenck U., Nieminen P., Jordan J., McGoogan E. *et al.* (2007b). European guidelines for quality assurance in cervical cancer screening: recommendations for collecting samples for conventional and liquid-based cytology. *Cytopathology* **18**: 133-9.

Arbyn M., Raifu A.O., Autier P. & Ferlay J. (2007c). Burden of cervical cancer in Europe: estimates for 2004. *Ann. Oncol.* **18**: 1708-15.

EXECUTIVE SUMMARY

Boyle P., Autier P., Bartelink H., Baselga J., Boffetta P., Burn J. *et al.* (2003). European Code Against Cancer and scientific justification: third version (2003). *Ann.Oncol.* **14**, 973-1005.

Coleman D., Day N., Douglas G., Farmery E., Lynge E., Philip J., & Segnan N. (1993). European guidelines for quality assurance in cervical cancer screening. Europe against cancer programme. *Eur J Cancer* **29A Suppl 4**, S1-S38.

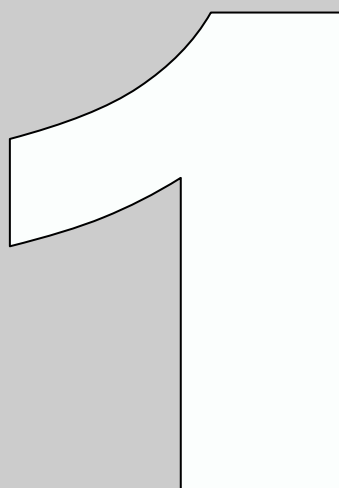
Council of the European Union (2003). Council Recommendation of 2 December on Cancer Screening. *Off J Eur Union* **878**, 34-38.

Herbert A., Bergeron C., Wiener H., Schenck U., Klinkhamer P., Bulten J., *et al.* (2007). European guidelines for quality assurance in cervical cancer screening: recommendations for cervical cytology terminology. *Cytopathology* **18**: 213-9.

International Agency for Research on Cancer (2005). Cervix Cancer Screening. IARC Handbooks of Cancer Prevention, Vol. 10. IARCPress, Lyon.

Miller A.B. (1993). Cervical cancer screening programmes: Managerial guidelines. World Health Organization, Geneva.

Wiener H.G., Klinkhamer P., Schenck U., Arbyn M., Bulten J., Bergeron C. *et al.* (2007). European guidelines for quality assurance in cervical cancer screening: recommendations for cytology laboratories. *Cytopathology* **18**: 67-78.



Introduction

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1.1 Burden of cervical cancer in the EU

Cancer is after cardiovascular disease the second most important cause of death in the European Union (EU) and is responsible for one in four deaths. Cancer is common in older people but cancer of the uterine cervix primarily affects younger women, with the majority of cases appearing between the ages of 35 and 50, when many women are actively involved in their careers or caring for their families (Gustafsson *et al.*, 1997). For the year 2004, the International Agency for Research on Cancer estimated that cervical cancer was diagnosed in approximately 34 300 women in the 27 member states of the European Union and about 16 300 women died from the disease (Arbyn *et al.*, 2007a & b; Boyle & Ferlay, 2005). Within the EU, wide variation is observed between countries with high and low mortality. The mortality was highest in Romania and Lithuania (world standardised rates of 13.7 and 10.0/100,000 women/year, respectively) and lowest in Finland (1.1/100,000/year). The burden of cervical cancer is particularly high in the new member states. With the exception of Malta, all 11 other newly acceded members have higher incidence and mortality rates for cervix cancer than the 15 countries belonging to the European Union before the expansion in 2004 and 2007. The east-west contrast is obvious in the map in Fig. 1, which shows the geographical distribution of mortality based on the estimates for 2004.

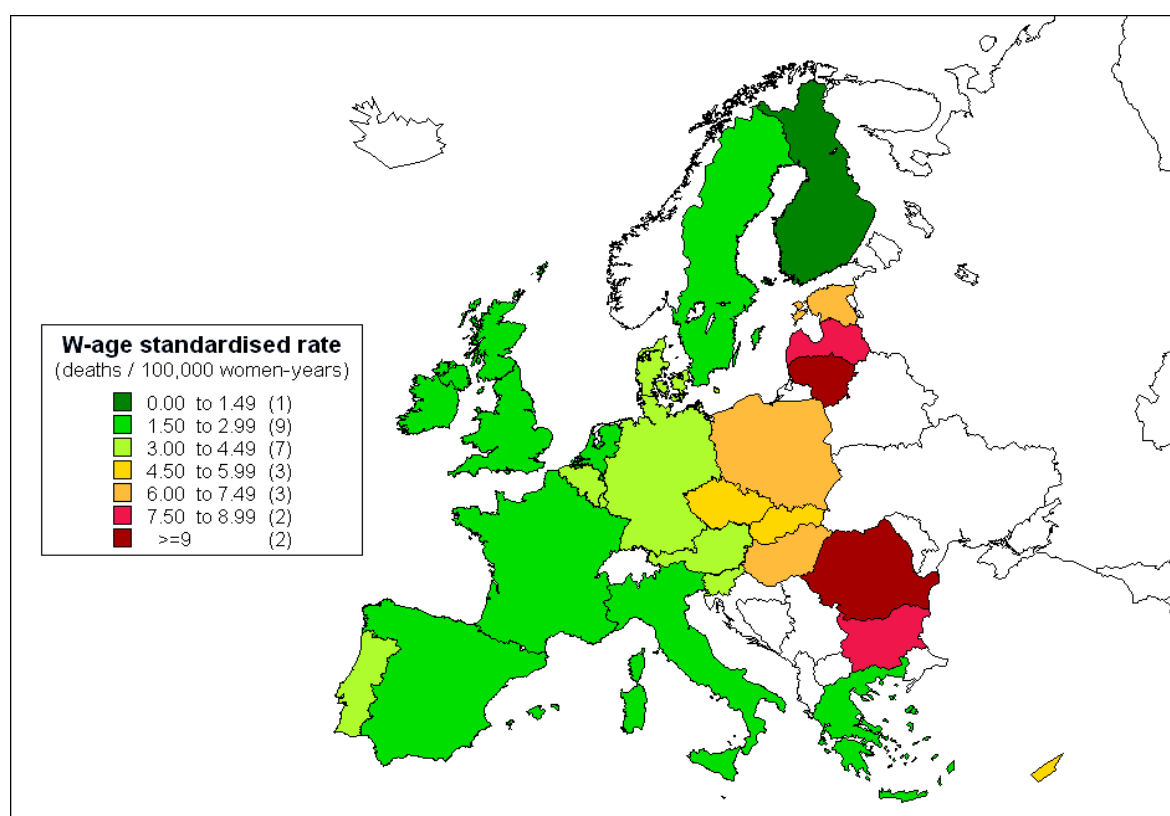


Fig. 1. Cervical cancer mortality in the 27 member states of the European Union, (world age-standardised rates, estimates for 2004). Adapted from: Arbyn *et al.*, 2007a & b.

1.2 Cervical cancer and screening

Among all malignant tumours, cervical cancer is the one which can be most effectively controlled by screening. Detection of cytological abnormalities by microscopic examination of Pap smears, and subsequent treatment of women in which cytological abnormalities are high-grade, avoids development of cancer (Miller, 1993). Well organised cytological screening at the population level, every three to five years, can reduce the incidence up to 80% (IARC, 2005). In industrialized countries, incidence of and mortality from cervical cancer has declined dramatically, most probably as a consequence of cytological screening (Bray *et al.*, 2005; Devesa *et al.*, 1987). However, cytological screening is only well organized in a few countries, such as the Nordic countries, the United Kingdom, the Netherlands and parts of Italy (Anttila *et al.*, 2004). In most other countries, screening is opportunistic, depending on the initiative of the individual woman or her doctor. Such opportunistic screening is most often characterised by a high coverage in selected parts of the population which are screened too frequently, coexisting with a low coverage in other socio-economically less developed population groups and heterogeneous quality, resulting in poor cost effectiveness (van Ballegooijen *et al.*, 2000; van den Akker van Marle *et al.*, 2002; Miller, 2002).

1.3 Cause of cervical cancer

Persistent infection with one of 13 to 16 oncogenic human papillomavirus (HPV) types is necessary but not sufficient for the development of cervical cancer (Muñoz *et al.*, 2003; Cogliano *et al.*, 2005). Recent data from cohort studies have shown that HPV 16 in particular has a high potential for malignant transformation of infected cervical cells (Schiffman *et al.*, 2005). The main route of HPV transmission is sexual. Cervical cancer without HPV is extremely rare (Walboomers *et al.*, 1999). Nevertheless, HPV infection is very common after onset of sexual activity and usually clears without any intervention. The factors that determine progression of HPV infection to high-grade cervical lesions and cancer are poorly understood. Co-factors for cervical cancer are: smoking, oral contraception, high parity, decreased immunity, including HIV infection and infection with Chlamydia trachomatis. The prevalence of HPV infection increased over the last decades and is probably responsible for the increased risk of cervical cancer observed among women born after the 1940s in most industrialised countries. The overwhelming evidence linking HPV infection to cervical cancer has prompted the development of several test systems to detect its nucleic acids and to develop prophylactic and therapeutic vaccines.

1.4 European policy: Council Recommendation of 2 December 2003 on Cancer Screening

In 2003, all national ministers responsible for public health in the member states of the EU, endorsed the scientific consensus which was reached by experts in cancer prevention (Council of the European Union, 2003; Advisory Committee on Cancer Prevention, 2000; Arbyn *et al.*, 2003). This recommendation constitutes a benchmark in the history of evidence-based cancer control in Europe. The Council of the European Union recognises that for three malignancies – cancer of the breast and the cervix in women and colorectal cancer in men and women – sufficient evidence exists to recommend population-based, organised screening (Wilson & Jungner, 1968; Council of

the European Union & Committee of Ministers, 1994) in all countries of the European Union. The Pap smear is the recommended standard test for cervix screening which should start in the age range 20 to 30. Screening should continue at 3-to-5-year intervals until the age of 60 (Advisory Committee on Cancer Prevention, 2000; Boyle *et al.*, 2003), or 65 (Coleman *et al.*, 1993; IARC, 2005). The upper limit should not be lower than 60 years (Advisory Committee on Cancer Prevention, 2000). Moreover, the Council of the European Union recommends that high quality should be assured at all steps of the screening process (invitation, screening, diagnostic confirmation and treatment of lesions, and follow up after treatment) and therefore screening should be offered in organised settings, whereas opportunistic screening should be discouraged. Monitoring systems, including linkage between appropriate databases should be set up in order to verify performance and impact. Furthermore, high population coverage should be achieved. Screening can be further improved by introducing certain new methods, but this should only be done after thorough evaluation of effects and cost effectiveness using appropriate solid scientific study designs. Evidence regarding new techniques should be regularly pooled and updated.

1.5 First edition of the European Guidelines for Quality Assurance in Cervical Cancer Screening

In 1993, the first edition of the guidelines for cervical cancer screening, was published in a synthetic format in the European Journal of Cancer (Coleman *et al.*, 1993). This edition established the principles of organised screening which are still valid today. It was pivotal in initiating new pilot projects in Europe and pioneering in launching the concept of quality assurance. Nevertheless, the 1993 version has had limited impact on opportunistic screening in countries with a 'liberal' health care policy. The second edition contains much more technical details and documentation. In particular, it provides a comprehensive and up to date overview of three new technologies: liquid-based cytology, automated interpretation of Pap smears and last but not least testing for human papillomaviruses. In addition, the current guideline has been extended with comprehensive instructions for general practitioners, gynaecologists and cytopathologists, prepared by a multi-disciplinary team of experts.

1.6 Content of the second guideline edition

The main body of the second edition of the guideline consists of seven chapters, beginning with the Introduction. The natural history of precursor lesions and cervical cancer, the epidemiological scientific basis for cytology-based screening and the principles for defining, implementing and evaluating evidence-based screening policy are covered in Chapter 2. Different screening systems and possibilities of articulation between organised and opportunistic screening activities are also discussed. The annex to Chapter 2 contains a series of basic tables that are useful to describe the main components of a screening system in place at the regional or national level and which allow computation of performance indicators. In Chapter 3, the current knowledge of the test characteristics of the conventional Pap smear and also of two newer methods for preparation (liquid-based cytology) or interpretation (automated devices) of cervical smears is synthesised. Colposcopy is only briefly described since it is not an appropriate screening tool. Chapter 3 ends with a review of three possible clinical applications of HPV testing: screening, triage of women with equivocal or low-grade

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Pap smear results and follow-up after conservative treatment of cervical lesions. Chapter 3 includes two annexes: (1) a guideline on how to prepare an adequate cervical smear and (2) recommendations for cervical cytology terminology which permit reporting the cytological findings of a cervical smear or a liquid-based preparation according to uniform principles. Uniform grading of cellular abnormalities is an essential condition for registration and comparisons over time and between different settings. The next two Chapters, 4 and 5, deal with quality assurance, certification and training in laboratory practice in cytology and histology, respectively. In Chapter 6, guidance is provided for management of screen-detected lesions, including specific instructions for colposcopists and treatment. The final Chapter 7 includes recommended key performance indicators dealing with invitation, participation, screening, management and treatment of screen-positive women, most of which can be computed from the data tables annexed to Chapter 2. For some indicators, linkage with cancer registry data is required.

The success of a screening programme requires adequate communication with women, health professionals and persons responsible for the health care system. This is addressed in a special appendix. The current guideline edition deals with screening for cervical cancer precursors, including management of screen-detected lesions. However, it is expected that primary prevention by means of prophylactic HPV vaccination will also become available in the near future. Therefore an additional appendix has been included, with the newest results of vaccination trials and a summary of pending questions.

1.7 The future

In the near future, two newly established EU-funded networks will continue to collect information on how screening is implemented in Europe and how it can be improved. The European Network for Information in Cancer Epidemiology (EUNICE) will collect data on the different steps of the screening process from all member states as recommended in Chapter 2 and 7. EUNICE will assist in standardising data collection procedures and in training of epidemiologists. EUNICE will also complete the information on the current burden of cervical cancer and study how it is influenced by screening and risk factors. Another network, the European Cancer Network (ECN), will focus on further development and implementation of quality assurance guidelines for cancer screening in order, among other things, to contribute to dissemination of the current guidelines, to study how the guidelines are used in defining and implementing best practice in each member state, to share experiences among experts, to pool information on new screening and management procedures, and to provide assistance in piloting and implementing regional and national screening programmes.

The next few years will be particularly challenging for the future European policy for cervical cancer prevention. In 2007 and 2008, the results of some relevant outcomes of several ongoing European trials will be published, which compare cytology screening with HPV or combined HPV/cytology screening. Moreover, it is expected that in the near future prophylactic vaccines protecting against HPV16 and HPV 18 infection, will be licensed¹. Both HPV types are causally linked with approximately 70% of cervical cancers in Europe (Muñoz *et al.*, 2004; Clifford *et al.*, 2003). The vaccines which are currently evaluated in phase 3 trials aim to protect girls or young women not yet infected. This means that, for the next decades, generations having initiated sexual contacts will continue to require screening. Nevertheless, as future vaccinated cohorts grow older, screening policies may need modification. The ECN network will follow these new developments with particular attention and gather information relevant to future updates of the guidelines.

¹ Meanwhile, in September 2006, a quadrivalent vaccine, protecting not only against infection with HPV16 and HPV18 but also against HPV6 and HPV11 (which causes genital warts) has been licensed for marketing in the EU (see Appendix 2).

The current guideline edition was prepared by experts from member states before the expansion of the European Union in 2004 and 2007. It has already been mentioned that the burden of cervical cancer is substantially higher among 11 new member states. Although experts from 7 of the 11 new EU member states participated in final discussions of the content of the guidelines at the 2006 annual meeting of the ECN, it is unknown to what extent the current guidelines address potential special needs and capacities of these countries. Contacts through the ECN and EUNICE networks will be informative and useful in this regard. Nevertheless, European authorities and representatives of the European Parliament should be aware that the substantially higher dimension of this public health problem in the east of the EU requires special attention.

The Council recommendation recognizes the urgency for establishing organised screening programmes of requisite quality and calls for a progress report of the European Commission based on information provided by the EU member states before the end of 2007. We hope that the current guidelines will also assist health authorities to initiate organised screening wherever it may still be lacking.

1.8 Acknowledgements

Numerous persons, all of whom cannot be mentioned here, have contributed to the current guideline edition. Their dedication and support is gratefully acknowledged. Special thanks are due to the colleagues of the previous European Network for Cervical Cancer Screening, in particular Prof. Ulrich Schenck and his team, who coordinated the network from 1999 to 2003, and also the other invited experts, all of whom have worked extensively and with great enthusiasm on this guideline. Their commitment, advice, perseverance, wisdom and patience are also gratefully acknowledged. Special thanks are also due to the Health and Consumer Protection Directorate General of the European Commission which provided financial support and, in particular, to the responsible technical officer, Dr. Karl Freese, who inspired and encouraged the editors and co-authors. Furthermore, the support of the Director of the International Agency for Research on Cancer, Dr. Peter Boyle, for the final manuscript review and technical editing, and the financial support of the Cochrane Gynaecological Cancer Review Group (Bath, United Kingdom) is also gratefully acknowledged.

1.9 References

Advisory Committee on Cancer Prevention (2000). Recommendations on cancer screening in the European Union. Advisory Committee on Cancer Prevention. *Eur.J.Cancer* **36**, 1473-1478.

Anttila A., Ronco G., Clifford G., Bray F., Hakama M., Arbyn M., & Weiderpass E. (2004). Cervical cancer screening programmes and policies in 18 European countries. *Brit. J. Cancer* **91**, 935-941.

Arbyn M., Raifu A.O., Autier P., & Ferlay J. (2007a). Burden of cervical cancer in Europe: estimates for 2004. *Ann.Oncol.* **18**: doi: 10.1093/annonc/mdm079.

Arbyn M., Autier P., & Ferlay J. (2007b). Burden of cervical cancer in the 27 member states of the European Union: estimates for 2004. *Ann. Oncol.* **18**, 1425-7.

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Arbyn M., Van Oyen H., Lynge E., Micksche M., Faivre J., & Jordan J. (2003). European Commission's proposal for a Council recommendation on cancer screening. *BMJ* **327**, 289-290.

Boyle P., Autier P., Bartelink H., Baselga J., Boffetta P., Burn J., Burns H.J.G., Christensen L., Denis L., Dicato M., Diehl V., Doll R., Franceschi S., Gillis C.R., Gray N., Griciute L., Hackshaw A., Kasler M., Kogevinas M., KVINNSLAND S., La Vecchia C., Levi F., McVie J.G., Maisonneuve P., Martin-Moreno J.M., Newton Bishop J., Oleari F., Perrin P., Quinn M., Richards M., Ringborg U., Scully C., Siracka E., Storm H., Tubiana M., Tursz T., Veronesi U., Wald N., Weber W., Zaridze D.G., Zatonski W., & zur Hausen H. (2003). European Code Against Cancer and scientific justification: third version (2003). *Ann.Oncol.* **14**, 973-1005.

Boyle P. & Ferlay J. (2005). Cancer incidence and mortality in Europe, 2004. *Ann.Oncol.* **16**, 481-488.

Bray F., Loos A.H., McCarron P., Weiderpass E., Arbyn M., Moller H., Hakama M., & Parkin D.M. (2005). Trends in cervical squamous cell carcinoma incidence in 13 European countries: changing risk and the effects of screening. *Cancer Epidemiol.Biomarkers Prev.* **14**, 677-686.

Clifford G.M., Smith J.S., Plummer M., Muñoz N., & Franceschi S. (2003). Human papillomavirus types in invasive cervical cancer worldwide: a meta-analysis. *Brit J Cancer* **88**, 63-73.

Cogliano V., Baan R., Straif K., Grosse Y., Secretan B., & El Ghissassi F. (2005). Carcinogenicity of human papillomaviruses. *Lancet Oncol.* **6**, 204.

Coleman D., Day N., Douglas G., Farmery E., Lynge E., Philip J., & Segnan N. (1993). European Guidelines for Quality Assurance in Cervical Cancer Screening. Europe against cancer programme. *Eur.J.Cancer* **29A Suppl 4**, S1-S38.

Council of the European Union & Committee of Ministers. Recommendation Nr R(94)11, on screening as a tool of preventive medicine. Strasbourg: Oct. 10, 1994.

Council of the European Union (2003). Council Recommendation of 2 December on Cancer Screening. *Off J Eur Union-* **878**, 34-38.

Devesa S.S., Silverman D.T., Young J.L., Pollack E.S., Brown C.C., Horm J.W., Percy C.L., Myers M.H., McKay F.W., & Fraumeni J.F. (1987). Cancer incidence and mortality trends among whites in the United States, 1947-84. *J.Natl.Cancer Inst.* **79**, 701-745.

Gustafsson L., Ponten J., Zack M., & Adami H.-O. (1997). International incidence rates of invasive cervical cancer after introduction of cytological screening. *Cancer Causes Control* **8**, 755-763.

IARC (2005). Cervix Cancer Screening. IARC Handbooks of Cancer Prevention. Vol. 10. IARC Press, Lyon

Miller A.B. (1993). Cervical cancer screening programmes. Managerial guidelines. World Health Organisation, Geneva.

Miller A.B. (2002). The (in)efficiency of cervical screening in Europe. *Eur.J.Cancer* **38**, 321-326.

Muñoz N., Bosch F.X., Castellsague X., Diaz M., de Sanjose S., Hammouda D., Shah K.V., & Meijer C.J. (2004). Against which human papillomavirus types shall we vaccinate and screen? The international perspective. *Int.J.Cancer* **111**, 278-285.

Muñoz N., Bosch F.X., de Sanjose S., Herrero R., Castellsague X., Shah K.V., Snijders P.J., & Meijer C.J. (2003). Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N.Engl.J.Med.* **348**, 518-527.

Schiffman M.A., Herrero R., Desalle R., Hildesheim A., Wacholder S., Rodriguez A.C., Bratti M.C., Sherman M.E., Morales J., Guillen D., Alfaro M., Hutchinson M., Wright T.C., Solomon D., Chen Z., Schussler J., Castle P.E., & Burk R.D. (2005). The carcinogenicity of human papillomavirus types reflects viral evolution. *Virology* **337**, 76-84.

van Ballegooijen M., van den Akker van Marle M.E., Patnick J., Lynge E., Arbyn M., Anttila A., Ronco G., & Habbema D.F. (2000). Overview of important cervical cancer screening process values in EU-countries, and tentative predictions of the corresponding effectiveness and cost-effectiveness. *Eur.J.Cancer* **36**, 2177-2188.

van den Akker van Marle M.E., van Ballegooijen M., van Oortmarssen G.J., Boer R., & Habbema J.D.F. (2002). Cost-effectiveness of cervical cancer screening: comparison of screening policies. *J.Natl.Cancer Inst.* **94**, 193-204.

Walboomers J.M., Jacobs M.V., Manos M., Bosch F.X., Kummer J.A., Shah K.V., Snijders P.J., Peto J., Meijer C.J.L.M., & Muñoz N. (1999). Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J.Pathol.* **189**, 12-19.

Wilson J.M.G. & Jungner G. (1968): Principles and practice of screening for disease. Public Health Papers 34, 1. Geneva, World Health Organisation.

2

Epidemiological guidelines for quality assurance in cervical cancer screening

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2.1 Executive summary

The objective of screening for cervical cancer is to reduce the mortality and incidence of the invasive disease. There is extensive and strong evidence from well-organised cytological screening programmes that this objective can be realised. Organised screening for cervical cancer is run in several countries of the European Union, though the screening organisation, policies and practices vary considerably between member states, however.

In 2004, approximately 52,000 new cases of cervical cancer were diagnosed in the whole of Europe, and about 27,000 women died from the disease. There was approximately five-fold variation in the incidence rates between countries with the lowest and highest burden of the disease. The rates were particularly high in countries in the Eastern or Central European region, including several new member states, where screening programmes have not been implemented. If an optimal screening policy and organisation could be achieved in Europe, the levels of cervical cancer cases and deaths could substantially decrease.

To maximise the positive impact and minimise the adverse effects, screening should only be provided in organised settings. Designing a cervical cancer screening programme includes defining the screening policy, i.e., determining the target age group and the screening interval between normal test results (3 or 5 years), choosing the screening test systems, and establishing follow-up and treatment strategies for screen-positive women. The screening policy should take into account the variation in background risk in target populations and the natural history of the disease, which is characterized by a rather long detectable pre-clinical period and substantial regression rates of the pre-cancerous lesions. Moreover, a well-organised screening programme must reach high population acceptance and coverage, and must ensure and demonstrate good quality at all levels.

Population-based information systems need to be established for continuous monitoring of screening process indicators. An appropriate legal framework is required for registration of individual data and linkage between population databases, screening files, and cancer and mortality registers. The information system is an essential tool for managing the screening programme; computing the indicators of attendance, compliance, quality and impact; and providing feedback to involve health professionals, stakeholders and health authorities.

The programme design must permit evaluation. An experimental design that is suitable for evaluation of new screening policies in organised settings is recommended. It must be stressed, as stated above, that reducing incidence and mortality is the objective of cervical cancer screening. An observation that a new screening method detects more precursor lesions than the standard Pap smear does not demonstrate improved effectiveness. Further evidence is required to ascertain whether application of the new screening method results largely in over-diagnosis and over-treatment of non-progressive disease.

The aim of these epidemiological guidelines is to characterise the basic organisational structure of cervical cancer screening programmes, and to recommend a common methodology for their design, evaluation and reporting. These guidelines are particularly relevant to planning new cervical cancer screening programmes in Europe. Different solutions fulfilling the recommended methodological standards need to be implemented in different countries and regions with diverse levels of resources and general healthcare infrastructure.

2.2 Introduction

For the past 60 years, the Papanicolaou smear test has been used to screen for pre-cancerous lesions and early invasive squamous cell cancer in asymptomatic women. This test involves removing a sample of cells from the cervical epithelium, and examining their morphology under the microscope in order to identify abnormal cells. Depending on the severity of the detected cytological lesions, women will need to be investigated further with repeat cytology, colposcopy and histology, and treatment may be required.

The main objective of screening for cancer is to reduce mortality from the disease. In cervical screening, reducing the incidence of invasive disease is also an objective because pre-cancerous lesions are detected and treated. Currently, there is strong evidence that organised cervical cancer screening can reduce incidence and mortality (Hakama, 1982; Hakama *et al.*, 1986; Laara *et al.*, 1987; Sankila *et al.*, 2000; IARC, 2005).

Organised screening programmes for cervical cancer exist in several countries of the European Union. The screening policies, organisation and practices vary between countries (Linós & Riza, 2000; Anttila *et al.*, 2004; IARC, 2005). The same applies to effectiveness and cost-effectiveness (van Ballegooijen *et al.*, 2000; Miller, 2002). Inefficiency may derive from: (1) sub-optimal distribution of screening tests, leaving substantial proportions of women without any or regular screening tests, whereas others may be screened with unnecessarily short intervals, even when they have been proved healthy; and (2) sub-optimal professional quality and standards of screening. To maximise the positive impact and minimise potential adverse effects, it is recommended that screening be offered in organised settings (European Commission, 2003; Council of the European Union, 2003).

A concern is the scope and completeness of recorded information. The information system required to run a screening programme can be composed of several components, depending on the health services organisation. Reliable cancer registration is important. Links between individual data at the population, screening, cancer registry and treatment level are needed.

As with any public health policy, the design of a screening programme should permit its evaluation. Evidence of the effectiveness and quality of each national programme is required. Results of screening performance which make clear to decision-makers, staff, those invited to and attending screening, and the general public how well the programme is running should be published regularly. Other key components in the monitoring and evaluation of screening include: scientific evaluation of the effectiveness and outcomes of the screening programme based on established epidemiological methods; and ascertainment of, and feed-back of information on invasive cancers detected during or subsequent to screening.

The effectiveness of an organised screening programme is a function of the quality of its individual components. Epidemiology provides instruments that permit planning, guidance and evaluation of the entire process of a screening programme, from the organisational and administrative aspects up to assessment of the impact. The aim of the present epidemiological guidelines is to characterise the basic organisational structures of a screening programme, and to recommend methodologies for its design, reporting and evaluation using commonly agreed terminology, definitions and classifications. There are only a few internationally recognised standards for programme organisation and evaluation. These guidelines will be helpful for setting up new cervical cancer screening programmes, which often are needed in countries with limited health care resources, and for improving existing screening programmes in Europe. Adherence to these guidelines will allow each programme to measure the outcome of its screening process.

This chapter concentrates on characteristics and evidence-based information available on the effectiveness of cervical cancer screening programmes using conventional cytology as the screening test. Information using incidence and mortality endpoints are currently available only for this test method. During the last decade, alternative screening technologies have emerged, making some rationale for changes in validity and organisation of a screening programme. No definitive information on effectiveness is available yet for these methods (IARC, 2005). These methods, and aspects relevant to their possible introduction into a screening programme, are discussed in further detail elsewhere, particularly in Chapter 3.

2.3 Epidemiology of cervical cancer

2.3.1 Burden of disease

2.3.1.1 Current incidence and mortality of cervical cancer

Each year in Europe, approximately 52,000 new cases of cervical cancer are diagnosed, and 27,000 women die from the disease (Arbyn *et al.*, 2007a & b; Boyle & Ferlay, 2005); 34 300 of the incident cases and 16 300 deaths were estimated to occur in the current 27 member countries of the EU (Arbyn *et al.*, 2007b). The age-standardised rates, estimated for 2004 for all countries of the European Union and using the world standard population as reference, are shown in Fig. 1. There is about a five-fold variation in the national incidence rates across countries. Variation in the death rates from the disease appears even larger. Due to the problems in the accuracy of death certificates related to cancer of the uterus, with many deaths recorded as 'uterus cancer, not otherwise specified' (ICD-9 179), adjustments for this mis-classification have been made in the above figures. However, these adjustments may be problematic if the proportion 'uterus unspecified' is large.

2.3.1.2 Trends

Of particular importance for demonstration of the effect of organised screening are the data on time trends in the incidence of invasive cervical cancer and cervical cancer mortality in the Nordic countries (Hakama, 1982; Laara *et al.*, 1987) where reliable national data are available from the period before screening programmes were implemented.

Towards the end of the 1960s Finland, Sweden and Iceland had nation-wide, organised screening programmes, and the same was true for several Danish counties. Norway in contrast had organised screening in only a single county. From the mid 1960s a decrease was seen in both the incidence and mortality from cervical cancer in Finland, Sweden, Iceland and Denmark. The decrease compared with time before screening was largest in Finland (Hristova & Hakama, 1997; Anttila & Laara, 2000) where the age-standardised mortality rates decreased over 80% from the level of 7.0 deaths per 100,000 in early 1960s to 1.2 deaths per 100,000 in the 1990's (rates adjusted for age to the world standard population). At the start of the Finnish programme, women aged 30 to 54 years were invited at a five-year screening interval, and it was only in the early 1990s that the age groups 55 to 64 were added to the programme. All Finnish municipalities followed the invitational programme (Anttila *et al.*, 1999). In Sweden and Denmark, which have partially organised programmes, the mortality rate decreased by 52 and 66% respectively.

In Norway, the incidence increased until the mid-1970s, and the decrease in mortality was considerably less (40%) than in the other Nordic countries (see Fig. 2). At that time, opportunistic screening was frequent in Norway but an organised cervical cancer screening programme did not commence in Norway until 1995. (Hakama, 1982; Sankila *et al.*, 2000; Nygard *et al.*, 2002; IARC, 2005).

The incidence in the younger age group varies between countries. For example, in Finland, in the pre-screening period the incidence at age 30-34 years was 8 per 100,000 whereas it was 30 per 100,000 in Denmark (see Fig. 3). There may also be different registration or diagnostic criteria, e.g., in the proportion of micro-invasive cervical cancer cases. These differential risks need to be considered when planning the screening policy.

Further evidence of the impact of organised screening on cervical cancer mortality and incidence has developed in the United Kingdom. Cytological screening was introduced in the 1960s, but an organised programme including a call/recall system and quality assurance was not initiated until 1988. In the preceding years, mortality and incidence decreased by 1-2% per year, whereas since 1988 the decrease has been about 7% per year, despite an increased underlying risk of disease in women born since 1940 (Sasieni *et al.*, 1995; Quinn *et al.*, 1999; Sasieni & Adams, 1999; Peto *et al.*, 2004; Bray *et al.*, 2005; IARC, 2005). The mortality rate from cervical cancer increased in the Republic of Ireland in the absence of a screening programme in the period 1970-2000, whereas a decrease was observed in the UK and in Northern Ireland (Comber & Gavin, 2004).

Decreases in the range of 10-60% have also been observed in cervical cancer incidence or mortality in some countries with less organised, or opportunistic screening (Anttila & Laara, 2000; IARC, 2005). In trend studies, there are several limitations in separating screening effects from other factors influencing cervical cancer rates (for a discussion of the caveats see IARC, 2005). Using very old hospital sources for incidence data is problematic because of the potential inclusion of pre-invasive cases (CIN3) in the cancer series. To avoid this potential bias, the old data should be re-evaluated (Anttila & Laara, 2000). Use of 'cancer of the uterus, not otherwise specified' (NOS) as a stated cause of death has been common in many countries. This affects comparability over time. In Belgium, an attempt has been made to estimate the historical proportions of deaths ascribed to cancer of the uterus NOS that should be redistributed (mathematically) to the cervix (Arbyn & Geys, 2002). The corrected (estimated) age-standardised mortality rates decreased from 14 per 100,000 in the 1950s to 4.5 in the 1990s (68% decrease) while the certified rate decreased from 6.3 to 3.0 (52% decrease). Because screening has been often practiced for decades, and historical information on the intensity and quality of screening is not available, it is impossible to precisely estimate the risk of cervical cancer in the absence of screening for the whole Europe.

In contrast with the above developments, cervical cancer mortality or incidence rates are currently rising, notably among recent generations, in some Eastern and Northern European countries such as Bulgaria, Romania and Estonia, in which rates have historically been quite stable and in which documented screening activity has been lacking (Bray *et al.*, 2005; IARC, 2005).

The time-trend studies demonstrate that full implementation of organised screening in Europe would lead to substantial decrease in current levels of cervical cancer incidence and mortality. Large decreases can be achieved in countries currently lacking screening, but cancer rates may also be expected to decrease in areas previously served only with opportunistic screening.

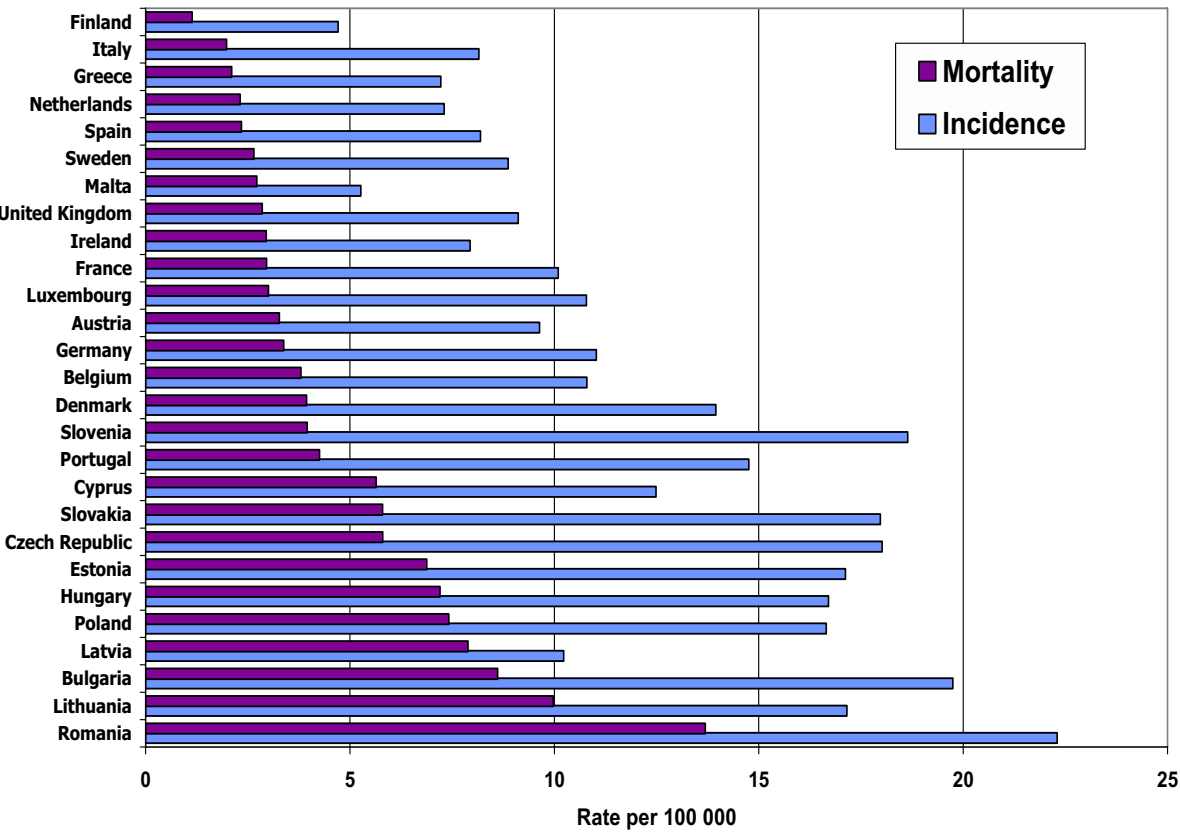


Fig. 1. Age-standardised rates of incidence of and mortality from cervical cancer (/100,000 women-years) in the 27 member states of the European Union, ranked by increasing mortality, estimates for 2004 (direct standardisation using the World reference population). (derived from Arbyn *et al.*, *Ann Oncol.* 2007b).

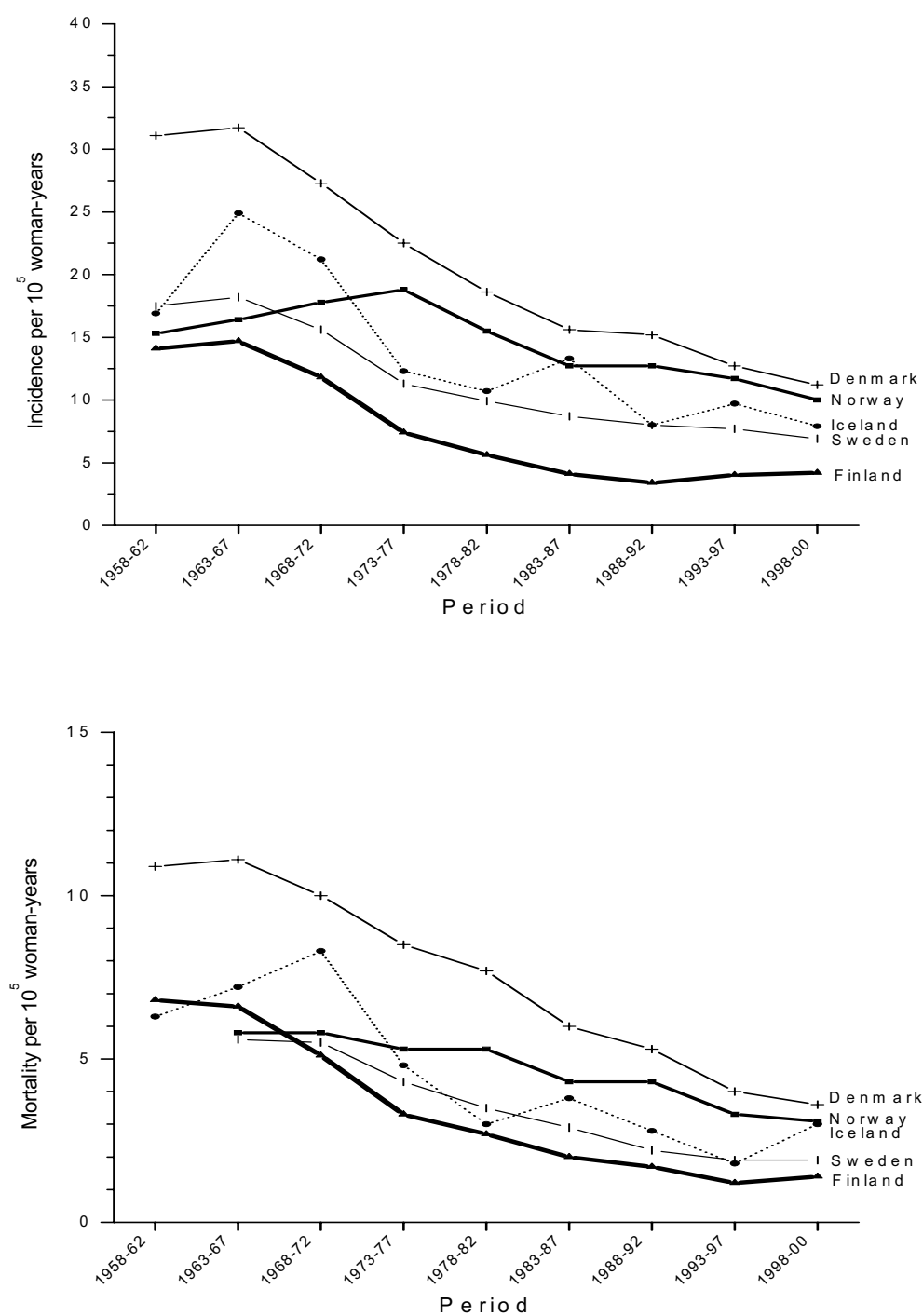


Fig. 2. Incidence and mortality rates of cervical cancer in the Nordic countries, 1958-2000. Adjusted for age to the world standard population

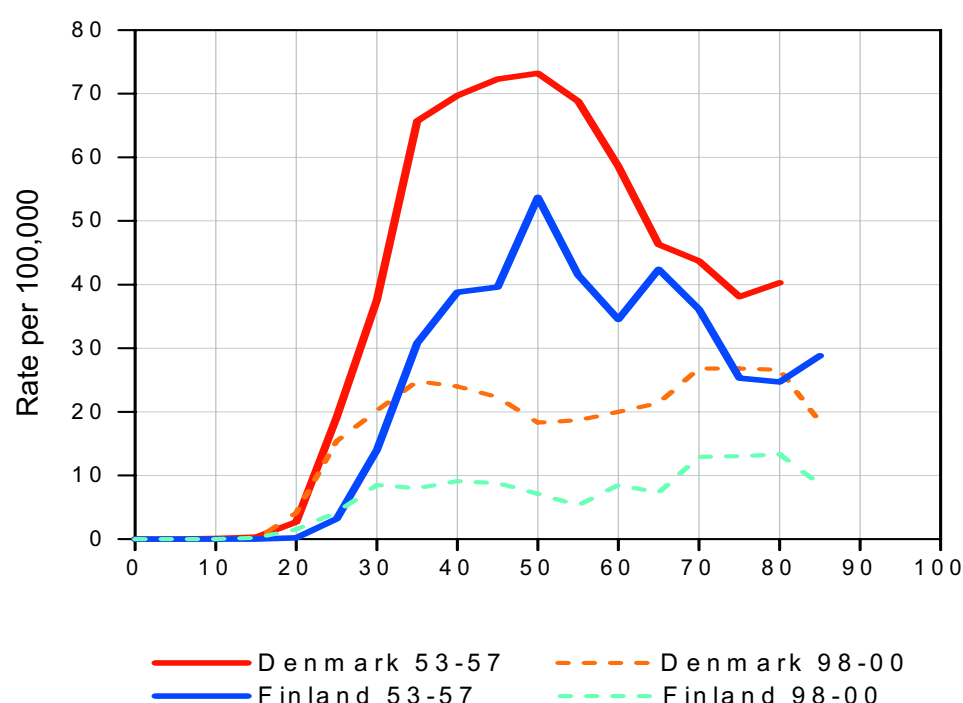


Fig. 3. Cervical cancer in Denmark and Finland, incidence by age

2.3.1.3 Survival

The relative 5-year survival rate of cervical cancer was 62% in cancers diagnosed in 1990-1994 in the EUROCare-3 (Sant *et al.*, 2003). There was some tendency towards a lower survival rate in Eastern Europe (Poland 48%, Estonia 53%, Slovakia 57%), while there was little variation in other parts of Europe. In EUROCare 4 the average was 60% in cancers diagnosed in 2000-2002, and the survival estimate in Eastern European countries (Czech Republic, Poland) was somewhat lower than the averages in other parts of Europe (Verdecchia *et al.*, 2007). Comparability of survival estimates between countries is a controversial issue; this is due to variation in the completeness of follow-up, and lack of comparable staging information (Berrino, 2003). A screening programme will introduce lead-time bias causing over-estimation of survival (Berrino, 2003). A cervical cancer screening programme may, on the other hand, prevent slow-growing tumours more than fast-growing tumours, introducing a controversial trend in the survival rate in a very long-term analysis (Dickman *et al.*, 1999). Since survival outcome is related to stage of cancer at time of diagnosis, screening may affect mortality more than incidence, by increasing detection of slow-growing cancers in asymptomatic women. The majority of these lesions are usually stage 1b or microinvasive. The differences in survival results between countries indicate, however, that emphasis on high-quality cancer treatments throughout Europe is in a priority.

2.3.2 Natural history of disease

Decisions on the optimal age group and screening interval require information on age-specific rates and on the duration of the period before the onset of invasion during which precursor lesions are detectable. It is now clear that many of these lesions, particularly mild dysplasia (CIN1) will not progress, and will in fact regress. Substantial variation in the progression and regression rates of pre-cancerous lesions has been reported (Östör, 1993) in a review of previously published papers. In this review Östör proposed that only a very small fraction of CIN1 and CIN2 lesions, 1% and 5% respectively, would progress to invasive cancer if untreated, whereas over 12% of CIN 3 lesions were estimated to do so (Table 1). Caution is needed in interpreting the estimates (IARC, 2005). Length of follow-up varied from <1 to 20 years in the studies included in Östör's review, and age groups may have varied; these numbers may therefore underestimate progression rates in screening populations.

A modelling study using data from the screening programme in British Columbia, Canada, showed that regression of pre-cancerous lesions vary by age: from 84% at age 18-34 years to 40% at 35 or more years (van Oortmarssen & Habbema, 1991). The estimated progression rates were 16% and 60%, respectively. No distinction was made between high- and low-grade lesions. In another early study on the British Columbia data, the progression rates of any CIN was estimated to be in the range of 19% to 38% (Boyes et al., 1982; IARC, 2005). An early Finnish study estimated that 28% to 39% high degree (severe) dysplasia and carcinoma in situ cases combined will progress to invasive cancer (Hakama & Räsänen-Virtanen, 1976). This was based on comparisons of cumulative incidence in women aged 30 to 59 years using an average follow-up time of about 3.5 years. Studies using cytological diagnosis have found lower progression rates (IARC, 2005).

By modelling screening data on the detection rate of intraepithelial lesions and on the incidence of cancer after a negative cytology Gustafsson & Adami (1989) and van Oortmarssen and Habbema (1991) independently estimated the mean duration of the phase of intraepithelial lesions before progression to invasive cancer to be 12 years. Observational studies (Luthra et al., 1987; Syrjänen, 1996) have reported an average duration of about ten years (variation from 5 to 15 years) for a pre-cancerous lesion to develop into invasive disease if left untreated. Progression might sometimes take even up to 50 years, as there are cancers detected also in very old women (e.g., over 75 years).

Table 1. Suggested regression/persistence/progression likelihoods of pre-cancerous lesions (Östör, 1993)

Severity of the lesion	Regression	Persistence	Progression to CIN3	Progression to invasive cancer
CIN1	60%	30%	10%	1%
CIN2	40%	40%	20%	5%
CIN3	33%	<55%	--	>12%

2.3.2 Risk factors

Oncogenic HPV infections are the most important risk factor for cervical cancer. Risk ratios or odds ratios of cervical cancer for exposure have typically varied between 15 and 150 (IARC, 2005). From among over 130 different types of human papillomaviruses, nowadays some sixteen types have

been classified as high risk (see Chapter 3). There are several other risk factors or co-factors, such as tobacco smoking (OR in case-control studies typically between 2-5, also among HPV-infected; IARC 2005); use of hormonal contraceptives, parity (if >5 children), and other genital infections such as Herpes simplex type 2, Chlamydia trachomatis, and HIV. A very high parity is not a usual condition in European countries similarly than e.g. in many developing countries; there are also negative studies with rather low overall numbers of children. Increase in the cervical cancer risk in young generations born after 1940 (Bray *et al.*, 2002) suggests that the prevalence of risk factors has increased. HPV is mainly transmitted through sexual intercourse, even though some other routes are, in principle, possible. IARC (2005) has recently reviewed the information on the risk factors.

Epidemiologic and virologic studies indicate that HPV infection is characterized by a very high rate of acquisition as well as spontaneous clearance (IARC, 2005). The most important determinants of HPV acquisition are the age at first sexual intercourse and the lifetime number of sexual partners of the woman or her partner. Condom use has shown protection against HPV infection in some studies, but most studies have not shown a significant protection. Several reasons for the apparent lack of protection have been proposed, including incorrect or inconsistent use, and the fact that the HPV infection may exist in a wider area of genital epithelium than is covered by the condom (Rousseau *et al.*, 2003).

2.3.3 Evidence for efficacy and effectiveness of cytological screening

Pap smear screening was never evaluated in a randomised trial. Nevertheless, evidence of its effectiveness, derived from observational studies, is convincing. These are cohort studies involving follow-up of screened women (Hakama & Räsänen-Virtanen, 1976; Johannesson *et al.*, 1982; Berrino *et al.*, 1984; Hakama *et al.*, 1986; Lynge, 2000; IARC, 2005), case-control studies (Clarke & Anderson, 1979; Nieminen *et al.*, 1999; Zappa & Ciatto, 2000; IARC, 2005), as well as time trend studies and ecological or geographical correlation studies (Miller *et al.*, 1976; Hakama, 1982; Laara *et al.*, 1987; Engeland *et al.*, 1993; Sigurdsson, 1995; Hristova & Hakama, 1997; Sankila *et al.*, 2000; Anttila & Laara, 2000; IARC, 2005); their main results are discussed in section 2.3.1.2.

Some of the most convincing evidence is based on a multi-centre IARC study in which individual screening histories were linked to cancer registry data (Hakama *et al.*, 1986, see below). The study material included both cohort follow-up and case-control studies. The pooled results provide the basis for recommendations on how often women with negative smears should be re-screened. The study followed the incidence of squamous cell cervical cancer among women who at the age of 35 had had two negative smears. When considering the impact of screening policy on the target population, as in trend studies or in follow-up studies by invitational status, account should be taken of: selection bias between participants and non-participants (IARC, 2005); lead-time in subsequent screen-detected cancers (Miller, 2002); and the possibility that cancers may be detected in women with a positive screening test and negative or non-compliant assessment. Most studies on cervical screening policy using individual data are based on incidence. More studies on the effect of different screening policies using mortality outcomes are needed.

2.3.3.1 Age group to be targeted

A smear taken between 35 and 64 years of age is much more effective in detecting a progressive lesion than a smear taken at age 20 (see section 2.3.2). Table 2 illustrates the impact of different screening policies on cancer incidence, based on the follow-up of women with negative smears (from IARC, 1986). There was no additional impact of starting screening at age 20 compared to starting at age 25. Starting at age 30 was not reported. Evidence of a lower effect of screening below age 30 was suggested by a recent study from the UK (Sasieni *et al.*, 2003) (see below).

When planning to start a new programme, resources should be concentrated on the age range from 30 or 35 to 60 years. A good guide would be to start screening 5 years before the age at which the age-specific curve of cervical cancer incidence begins to peak (WHO, 1986). As indicated from the developments of the cancer rates in Finland and other Nordic countries, not all age groups need to be covered at once; the programme can be started with rather few age groups. High coverage should be the main target (see Tables 2 and 3).

There is no firm evidence for the optimal age to stop screening. Different studies have shown a low detection rate of high-grade lesions over the age of 40 in previously screened women. Studies using mortality outcome are recommended. However, women over the age of routine screening who have never been screened should be entitled to screening on request, reasonably until at least two negative tests have been obtained.

2.3.3.2 Screening interval

According to the IARC multi-centre study (1986), 93% of the expected cases of squamous cell carcinoma could be avoided with screening every year, 91% with screening every third year, and 84% with screening every fifth year (see Table 2). A negative Pap smear result is associated with a strongly reduced risk of cervical cancer for at least 5 years. However, the study did not adjust for selection in attendance.

Table 4 shows results of two recent studies on the invasive cervical cancer risk among women screened negative. One of the studies was a cohort follow-up study conducted in the Netherlands, where estimates without screening were obtained from trend analysis (van den Akker-van Marle *et al.*, 2003c). The other was a case-control study conducted in the UK (Sasieni *et al.*, 2003). In the UK study, screening at an interval of 3.5 years and more was not associated with protection women aged 20-39 years (odds ratio >1). There are differences between these studies with respect to the definition of a negative smear. Selection among women screened, estimation of background trend, as well as cytological criteria and quality of screening may have affected the estimation of relative risks (IARC, 2005). Sasieni's data were used to recommend 3-yearly screening in women 25-49 and 5-yearly in women 50-64 in the UK. The recommendation is still in line with current EU guidelines.

2.3.3.3 Screening modality: organised vs. opportunistic screening

The early reports of trends in cervical cancer incidence and mortality, discussed in section 2.2., showed a clear decrease in countries or areas that widely implemented organised screening programmes in comparison with countries with no or opportunistic screening only. Early cohort follow-up studies among those invited for screening (Hakama & Räsänen-Virtanen, 1976; Johannesson *et al.*, 1982; Magnus *et al.*, 1987; Lynge, 2000) have also indicated that the decrease in cervical cancer incidence was particularly pronounced among participants in organised screening. The trend and follow-up studies in the UK also demonstrate effectiveness of an organised screening activity. A case-control study in Finland indicated that the effect of participating in organized screening was about two-fold higher than the effect of spontaneous screening. In women attending only orga-

nized screening, the effect was 75% (OR of cervical cancer of 0.25: 95% CI 0.1-0.5) in women attending only spontaneous screening, the screening effect was 43% (OR 0.57, 95% CI 0.3-1.1) (Nieminen *et al.*, 1999). Most women had participated in both screening modalities, and the OR in this group of women was 0.27 (95% CI 0.29-0.75).

In Denmark a RR of 0.67 (95% CI, 0.61-0.73) for women aged 30-59 years in 1963-1982 was observed when comparing counties with and without organised screening (Lynge *et al.*, 1989). A 20% decrease in incidence of fully invasive cervical cancer was observed in Turin, Italy, among women invited to an organized program, compared with those not invited, after introduction of the programme in an area in which intensive opportunistic screening was previously conducted (Ronco *et al.*, 2005).

In conclusion, organised screening appears to be more effective and largely more cost-effective than opportunistic activity.

Table 2. The effectiveness of different screening policies. Proportionate reduction in incidence of invasive squamous cell carcinoma of the cervix uteri assuming 100% compliance (IARC, 1986). Assuming that a woman is screened negative at age 35 and that she had at least one negative screen previously

Screening frequency	Age group	Reduction in cumulative incidence (%)	Numbers of smears per women
Every year	20-64	93	45
Every 3 years	20-64	91	15
Every 3 years	25-64	90	13
Every 3 years	35-64	78	10
Every 5 years	20-64	84	9
Every 5 years	25-64	82	8
Every 5 years	35-64	70	6
Every 10 years	25-64	64	5

Table 3. Reduction in cumulative incidence of squamous cell carcinoma of the cervix uteri with different screening intervals and proportions of women screened aged 35-64 in comparison with expectation without screening (Hakama *et al.*, 1986).

Screening interval	Proportion of women screened	Reduction in cumulative incidence (%)	Average number of tests per woman in the population
1 year	20%	19	6
2 year	30%	28	4.5
3 years	40%	37	4
5 years	50%	42	3
10 years	80%	51	2.4

Table 4. Relative risk of invasive carcinoma of the cervix uteri since screening negative in comparison with expectation without screening in the Netherlands (van den Akker-van Marle *et al.*, 2003b) and in comparison with non-screened in the U.K. (Sasieni *et al.*, 2003)

Time since screening	Country & study		
	Netherlands (van den Akker-van Marle ME <i>et al.</i> , 2003a) ¹		
	Ages 35-64		
	RR (95% CI)		
0-6 months	0.12 (0.08-0.17)		
7-12 months	0.06 (0.03-0.10)		
1-2 years	0.08 (0.06-0.12)		
2-4 years	0.15 (0.11-0.19)		
4-6 years	0.20 (0.14-0.29)		
6-10 years	0.18 (0.11-0.30)		
	England (Sasieni <i>et al.</i> , 2003) ²		
	Ages 20-39	Ages 40-59	Ages 55-69
	OR (95% CI)	OR (95% CI)	OR (95% CI)
0-18 months	0.24 (0.16-0.37)	0.12 (0.08-0.18)	0.13 (0.08-0.22)
18-30 months	0.33 (0.21-0.51)	0.14 (0.08-0.22)	0.13 (0.07-0.23)
30-42 months	0.67 (0.43-1.04)	0.25 (0.16-0.40)	0.15 (0.08-0.26)
42-54 months	1.06 (0.65-1.72)	0.30 (0.18-0.50)	0.18 (0.09-0.34)
54-66 months	1.40 (0.75-2.62)	0.61 (0.34-1.09)	0.28 (0.14-0.57)
66-78 months	1.86 (0.88-3.93)	0.72 (0.36-1.43)	0.33 (0.14-0.79)
>6 years	2.37 (1.16-4.85)	0.69 (0.36-1.34)	0.55 (0.27-1.10)

¹ Invasive cervical cancer since two or more previous negative screenings, in comparison with expectation without screening

² Invasive cervical cancer since the last operationally negative smear

2.4 Organisation of cervical cancer screening

2.4.1 Principles of the determination of a screening policy

2.4.1.1 Decision to run a screening programme

There should be a national and governmental context for planning for cervical cancer screening (Miller, 1992; WHO, 2002). The programme needs political support, with funding, to proceed. It is essential that the programme is integrated into the health care system and is accepted by both the population and the persons currently earning their living from smear taking and reading.

In many European countries, cervical cancer early detection activity exists in some form, e.g., testing personally initiated women, associated with some other programme, or performed in the context of maternal health care, or as a component of private health care. It is unlikely that simply providing funds to increase existing activity will enable the programme or screening policy to be successful. In parallel with introducing the general principles of organised screening, governments should consider the possibility of not paying for unnecessary excess smears.

2.4.1.2 European screening policy

Implementation of organised screening programmes for cervical cancer has been recommended by the Council of the European Union (2003). According to the Council Recommendation, systematic implementation of cancer screening programmes requires an organisation with a call/recall system and quality assurance at all levels, and an effective and appropriate diagnostic, treatment and after-care service following evidence-based guidelines. Centralised data systems are also needed to run organised screening programmes. The Council Recommendation includes further guidance on implementation, registration, monitoring and evaluation, training, informing screening participants and introducing novel screening tests. In many countries, the European recommendations are not yet fulfilled (Anttila *et al.*, 2004; IARC, 2005).

Cervical cancer screening has been recommended for the age group from 25 or 30 years to 60 or 65 years (Advisory Committee on Cancer Prevention, 2000; Coleman *et al.*, 1993); the Council Recommendation states that screening should start no earlier than at 20 years and no later than at 30 years of age. There is no mention when to stop screening in the Council recommendation. According to the recommendation by the Advisory Committee, the upper limit should not be lower than 60 years. The first edition of the QA Guidelines recommend that an optimal screening programme should aim at the population aged 25 to 65 (Coleman *et al.*, 1993). According to IARC, women who always tested negative should cease screening once they attain age 65 (IARC, 2005). European countries show substantial variation in the screening interval and age range of target groups (see Table 5). Screening more frequently than every three years should be discouraged as it is only marginally more effective and is certainly not cost-effective (IARC, 2005). There is no firm evidence for the optimal age at which to start screening (section 2.2). An early start will imply treatment of many CIN which if untreated would never have progressed to invasive cervical cancer. A very late start will inevitably imply that some early invasive cancers are missed. A start at the age of 15 is clearly too early because the incidence of invasive cancer is virtually zero until the age of 20, and as the early start will lead to overtreatment.

The recommendations of the Advisory Committee, which was established by the Europe against Cancer programme, also stated that cervical cancer screening should be offered at least every fifth year, and if resources are available, every third year. The number of unnecessary treatments increases with a large number of smears per lifetime. With limited resources, screening every fifth year with high quality and high compliance is preferable to screening every third year at a proportionally lower coverage.

Table 5. Average age-standardised mortality from cervical cancers per 100,000 in 1995 (European standard population) related to the recommended screening policy in some countries of the European Union (van Ballegooijen *et al.*, 2000; Bray *et al.*, 2002)

	Mortality from cervical cancer (1995)	Target age group	Screening interval (years)	Smears per lifetime	Proportion of the population subjected to a formal programme (%)	Proportion of women screened in a 3- or 5-year period (%)
Austria	6.3	20+	1	50+	n.r.e.	n.r.e.
Belgium (a)	4.6	25-64	3	14	58	78
Denmark	6.3	23-59 (f)	3	13	90	75
Finland	1.7	30-60	5	7	100	93
France	4.6	25-64	3	14	<5	n.r.e.
Germany	5.5	20+	1	50+	90	80
Greece (b)	3.0	25-64	3	14	n.r.e.	n.r.e.
Ireland (c)	4.6	25-60	5	8	n.r.e.	n.r.e.
Italy	3.2	25-64	3	14	13	50
Luxembourg	1.6	15+	1	55+	n.r.e.	n.r.e.
Netherlands	2.7	30-60	5	7	100	77
Portugal (d)	6.3	20-64	3	16	n.r.e.	n.r.e.
Spain (d)	3.5	25-65	3	14	n.r.e.	n.r.e.
Sweden	3.7	23-60	3(e)	14	100	82
UK (England)	5.0	20-64	3 or 5	10-16	100	61

a) Policy related to the Flemish region of Belgium; b) Policy related to pilot studies; c) Policy planned for one region of the country; d) Policy for one region of the country only; e) 5-yearly at ages 50-60 years; f) corrected.

2.4.2 Integration within the healthcare system

Organised cervical cancer screening is a multi-step process including:

- Identification of the target population
- Recruitment of eligible women
- Collection of Pap smears
- Examination of the Pap smear and reporting
- Reassurance of women with normal smears and information on the timing of the next smear
- Recall of women with unsatisfactory/inadequate smears
- Follow-up of women with abnormal smears, i.e. diagnostic procedures and treatment if needed, including a fail-safe system to make sure this actually happens
- Registration, monitoring and evaluation of the entire programme.

In some countries, re-allocation of resources already used for screening activities will be sufficient to cover the entire target population within a defined screening interval. Different solutions can be proposed to implement organised cervical cancer screening (e.g., depending on whether opportunistic activity currently exists). In general, systems which have demonstrated effectiveness can be recommended, and additional aspects relevant to cost-effectiveness and minimisation of potential adverse effects need to be taken into account.

In Finland and in some regions of Italy, a general call system is applied (Anttila & Nieminen, 2000; Segnan *et al.*, 2000). In Finland the programme was introduced when opportunistic screening activity was not common. All women in the target population are invited at the agreed interval by the smear takers involved in the programme. The smears are processed and analysed in defined laboratories under quality control. Women with abnormal smears are managed according to guidelines. Monitoring covers the complete screening episode, and records are linkable to the cancer registry. The advantages are that all women have access to well-organised screening and information on the correct screening policy. The disadvantage is that no information is captured for opportunistic screening, and observance of quality standards in opportunistic screening cannot be verified.

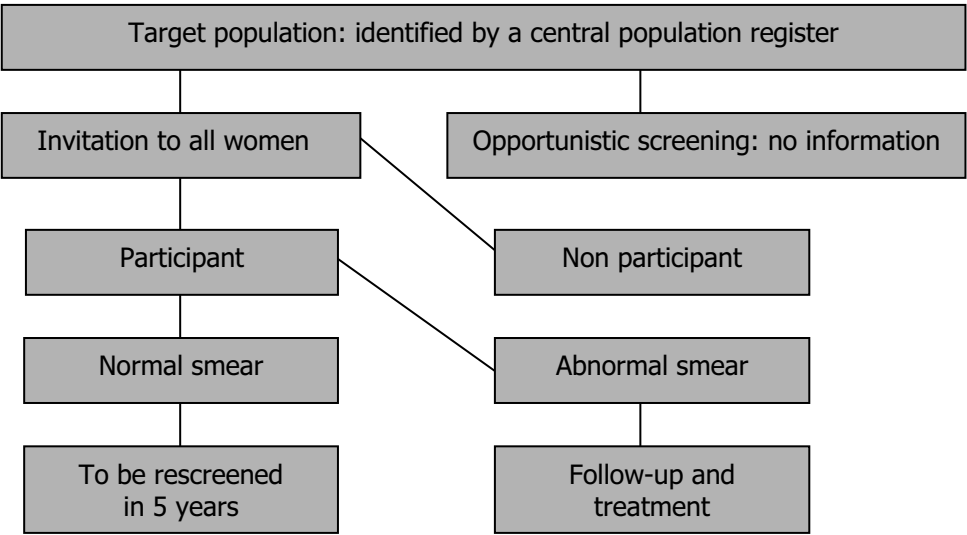


Fig. 4. Example of cervical cancer screening in Finland

All results of Pap smears, colposcopy, and histological interpretation of biopsies and information of treatment performed in the framework of organised screening are registered.

Call and recall screening programmes can be designed to make information on opportunistic smears available, as is the case in the Netherlands (van Ballegooijen & Hermens, 2000) and the United Kingdom (Patnick, 2000). In these countries, every woman in the target age group is invited at the appropriate interval to get a free programme smear (example of the Netherlands in Fig. 5). Non-attenders are identified by the laboratories and are reminded. Guidelines for quality assurance cover all steps of the screening process: smear taking, cytopathology and management of abnormal smears. In the Netherlands, every smear taken in the country is recorded in the PALGA (Dutch Network and National Database for Pathology) with the reasons for the smear (screening programme smear, opportunistic smear, repeat smear), the result, and recommendations on follow-up. All of these smears are subject to quality control by the laboratory. Opportunistic smears are not paid for, and their frequency has therefore decreased.

In the United Kingdom laboratories are required to inform the local health authority of the results of all smears: the health authority will then amend or change the date for the next recall. In the UK opportunistic screening is highly discouraged.

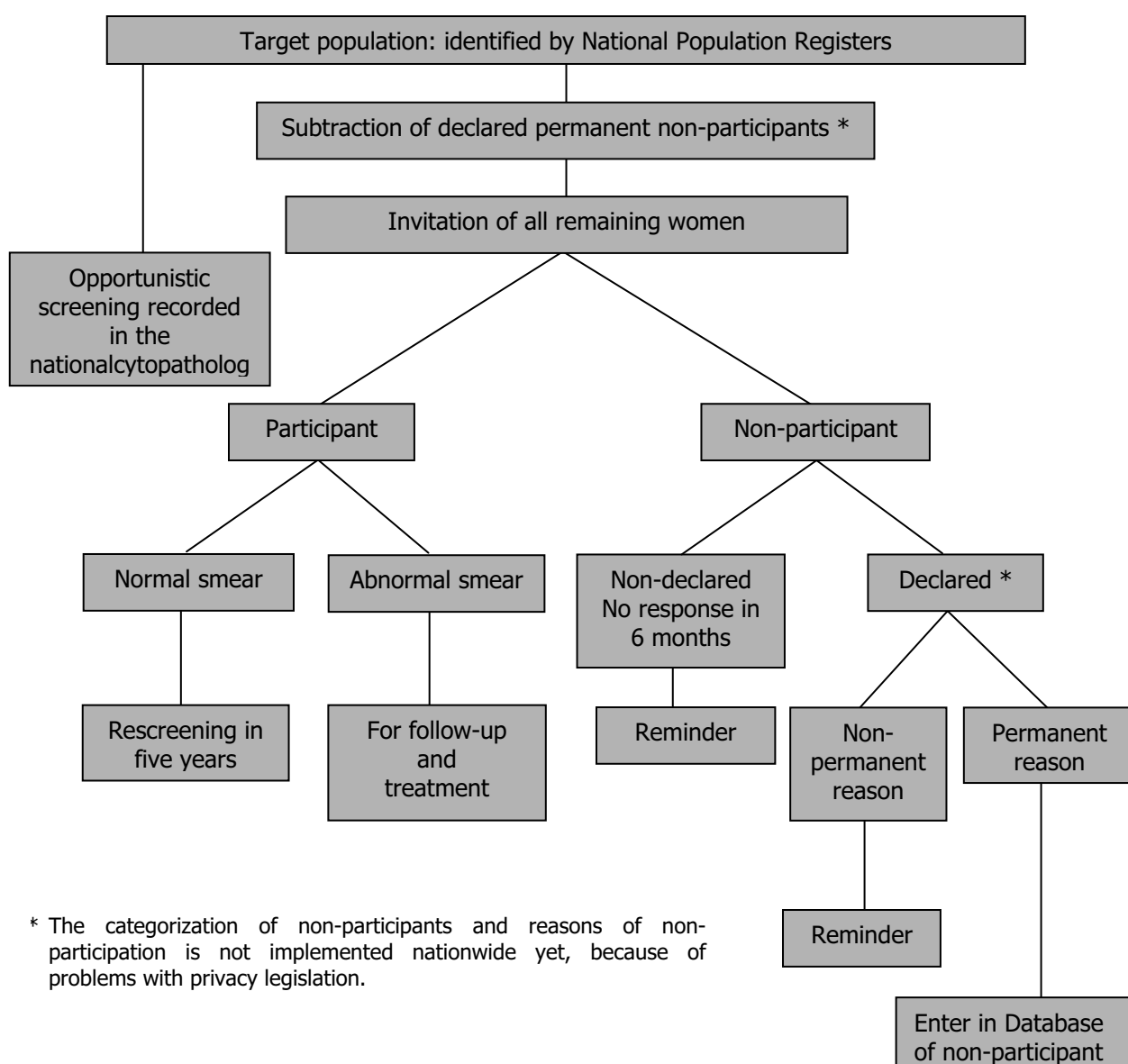


Fig. 5. Example of cervical cancer screening in the Netherlands

When opportunistic screening is already extensive, some countries only issue invitations to all women who have not had a smear taken within the screening interval in order to save resources. This call-recall system is followed in Denmark (Coleman *et al.*, 1993) (see Fig. 6) and Sweden (Dillner, 2000). This type of organisation is acceptable if opportunistic smears are subject to systematic quality control; otherwise, it produces ineffectiveness and inequalities. Information on recommended screening age and interval should be effectively disseminated to all women (not only to invited women) and smear takers, and excessive use of smears should be discouraged. Otherwise this system is expensive. A comprehensive evaluation and quality assurance activity should be integrated in the programme.

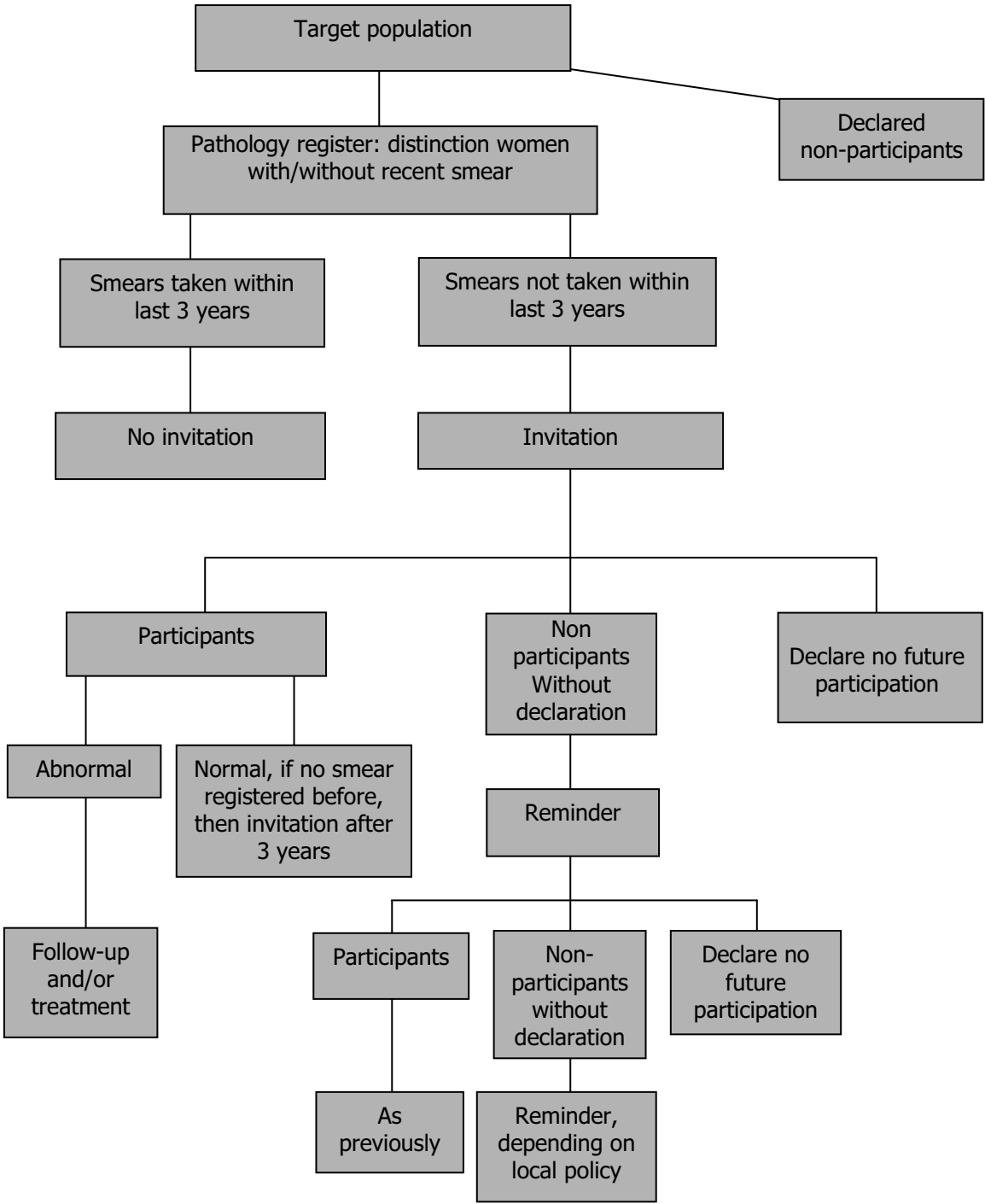


Fig. 6. Example of cervical cancer screening in Denmark

In some regions of France, a call-recall system is integrated into the health care system, in which screening remains essentially opportunistic (Fig. 7) (Schaffer *et al.*, 2000). Smears takers are recommended to follow the screening policy defined at a national consensus conference. All smears are registered, including the identification of the patient and the smear taker, the data of specimen collection and the result. All laboratories must have accepted the quality-assurance process and transmit computerised data on every smear. Laboratories have been compensated for the software needed for registration. The cost per smear is fixed by law. Guidelines for the management of abnormal smears are published and the follow-up outcomes are monitored. Fail-safe measures to avoid loss to follow-up are implemented. Personal letters are sent to all women who have not had a smear reimbursed by the health insurance system (which covers 80% of the population) within three years. No reminder is sent to non-participants. As this system is based on the voluntary collaboration of smear-takers to adhere to the recommended screening interval, a lot of unnecessary smears are still taken. However, quality of all smears and follow-up is under control. The participation rate is monitored and tools to increase compliance of the target population are implemented.

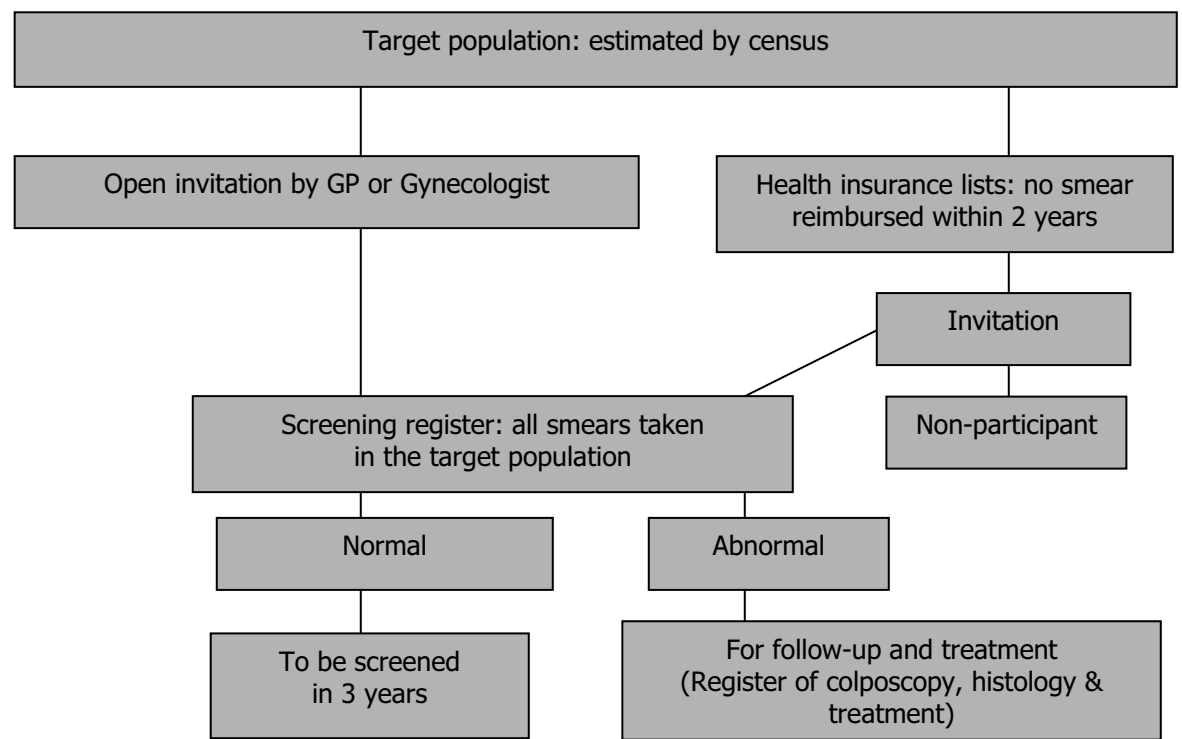


Fig. 7. Example of cervical cancer screening in Alsace (France)

2.4.2.1 Defining target population and relevant health-care professional and facilities

Before implementing the programme the target population must be clearly defined. It is necessary to describe the target population and to make a review of ongoing screening activities in the area covered by the target population. In order to run a successful programme, adequate resources, both in terms of staff and facilities must be available, and an adequate infrastructure must in place. Furthermore, the current situation and projections of the future burden of cervical cancer should be reported.

Owing to the diversity of the health systems and the diversity of the specific conditions in individual countries, the application of a single approach to organising quality assurance in all EU countries is

not a feasible target. However, guidelines on some crucial aspects are provided. Ideally, screening programmes should be implemented nationwide, but organized and managed locally, in each EU country.

Defining and describing the target population

As mentioned above, catchment areas and target populations must be clearly defined. An administrative centre should be identified for each area, and all resources necessary for the entire screening process should be present and well-inventoried. If all resources are not available in a given area, large centres, particularly for diagnosis and treatment, can serve more than one area, provided that adequate lines of communication are established. It is difficult to obtain adequate data for evaluation if a large proportion of smears are taken or reported, or biopsies are performed outside the respective catchment area. A high rate of migration will cause problems in the production of statistics. Stability of the population is therefore desirable and the population size of a catchment area should be large enough to ensure the stability of the statistics. Migration should be documented and changes in addresses regularly updated. For optimal administrative efficiency and stability of statistics, catchment areas with not less than 250,000 permanent inhabitants should be defined.

Identification of relevant health care professionals and facilities

Public health specialists

From the onset, public health specialists are needed to ensure that the programme includes a population-based information system that monitors each step of the screening process. They will then be responsible for gathering data and for ongoing monitoring in order to identify problems that need intervention. These public health specialists can be based at a national or regional level, whereas the other health professionals who are providing screening services are needed in each area. Public health specialists should have an understanding of basic epidemiology, statistics and communication training. A European training course on monitoring and evaluation of screening programmes would be desirable.

Smear takers and smear-taking facilities

Depending on each country's health system and culture, different health professionals can be involved in smear taking, i.e. physicians, nurses or paramedics. At present, GPs are very often the main smear takers in some EU countries, such as in Denmark and the Netherlands. Midwives or laboratory nurses play this role in Finland, Sweden, Italy and some pilot projects in Greece. Nurses can take smears well, as has been demonstrated in the UK. In Austria, Belgium, Germany and France most of the smears are taken by gynaecologists.

Each country should establish minimum training requirements for each type of smear-taker fulfilling the present European guidelines (see Appendix 1 of chapter 3). Smear takers should understand the anatomy of the female genital tract, the management of abnormal smear results and also the process of mass population screening. Smear takers must know how to use a speculum to visualise and assess the appearance of the cervix and must also understand the importance of sampling the transformation zone. They should be able to correctly interpret a report on a cervical smear.

It is important that women are satisfied with the service offered to them, or they will not return for re-screening or follow-up. Before the smear is taken, the environment for the taking of the smear should be suitable; there should be privacy, warmth and a relaxed atmosphere, and the woman must be comfortable.

Pathology laboratories

Laboratory guidelines for cervical screening and professional requirements for the staff (cytotechnologists and pathologists) are described in Chapters 4 and 5.

Diagnostic and treatment centres

Trained colposcopists are essential. Screening will not be efficient if abnormal smears are not followed by a proper evaluation of cervical lesions and appropriate management if needed. Each

national Colposcopy Society should establish a validated training course for colposcopy, following the guidelines in Chapter 6.

Informatics and office staff

Informatics and office staff are needed to issue invitations and register data at all steps of the process. They must be aware of the importance of confidentiality and accuracy in transfer of patient details.

Participation of GPs

Even if GPs are not taking smears, they should play an important role in the screening programme and be aware of how the programme is structured, and in particular, of the invitation scheme. They can advise non-compliers about screening, which is important for women who are no longer in contact with maternity or family planning services. The experience of the Netherlands and the UK demonstrates the effectiveness of GPs in this regard. GPs should be asked to have the date and result of each woman's last smear in her computerized medical file in order to advise her to have her smear at the appropriate time. GPs should receive a copy of the result of all smears performed for their patients.

GPs should also be aware that mortality rates are one of the important criteria to assess the effectiveness of screening programmes. They should know that an accurate certification of death is needed: "uterus otherwise unspecified" cancer should not be used on death certificates; the specific location of the cancer (endometrial or cervical) should always be given.

Coordination of the programme

As has been stated above, screening is an activity involving nurses, midwives, cytotechnicians, pathologists, gynaecologists, GPs and informatics and public health specialists. All these professionals need coordination. A committee in which all professionals are represented should be created to monitor and review local practice and policies and to ensure that they fit with regional or national policies and guidelines where they exist. The chairman should be appointed as the responsible programme manager.

Specific responsibilities should be assigned to the chairman for organisation, mass-media relationship, budget, quality assurance, evaluation etc, although he/she may need to delegate the execution of these functions to others. The programme manager should have necessary resources and authority to coordinate all professions and to implement the decisions of the committee. Whenever possible the consensus of all participants in the screening process should be obtained but without neglecting the aims of quality assurance.

Data infrastructures

An adequate information system is needed. A first need is an updated list of each member of the eligible target population (see below and section 2.3).

2.4.2.2 Inventory of baseline conditions

Before implementation of a screening programme, an inventory of baseline conditions comprising information on opportunistic screening should be made (see the "target population" section in the monitoring tables in the annex to this chapter). At a minimum, the information listed below in tables 7 and 8 should be collected. It is important to check at this time the feasibility of linkage between the cancer registry and the future screening registry because this is necessary for identification of interval cancers. In order to assess the effectiveness of the programme it is necessary to also measure outcomes in terms of reduced cervical cancer incidence and mortality.

Differences in reported cervical cancer incidence and mortality rates reflect, in addition to variation of background risk, also different diagnostic or registration criteria. For example, with regard to incidence data, it should be kept in mind that treatment of micro-invasive (FIGO stage Ia) cancers is

relatively conservative, and survival after treatment is very high. One should use incidence rates excluding the micro-invasive cancers (i.e., fully invasive only), if available. Another option to obtain relevant information, e.g., on the number of preventable deaths by age groups which may be targeted for screening is to calculate incidence-based mortality rates. This parameter accumulates deaths following from cervical cancers by age and/or calendar time of diagnosis, possibly also by length of follow-up. Respective parameters can be generated directly from individual data by linking incidence and death records. In the absence of the linkage, incidence-based mortality can be estimated from incidence and relative survival rates.

It is important that the exact topography of the cancer is mentioned on the death certificate when a woman dies from uterine cancer. Therefore, employees involved in vital statistics or the cancer registry should interrogate the medical officer who completed the death certificate if topographical information is missing. To produce reliable statistics, the percentage of uterine cancer deaths with known topography (cervix or corpus) should be at least 80%.

Table 6. Cancer registration in the target population

Details of the register	Cancer registry	Cervical cancer register	Ad hoc survey
National/Regional			
Overlap with screening area %			
Population based yes/no			
Accessible yes/no			
Microinvasive 1a registered separately			
CIN3 registered separately			

Table 7. Cervical cancer incidence per year/period

Age group	Population	Incidence		Mortality	
		Number of cases	Incidence rate (/100,000/y)	Number of deaths	Mortality rate (/100,000/y)
<25					
25-29					
30-34					
35-39					
40-44					
45-49					
50-54					
54-59					
60-64					
Add older age groups					
World ASR					

2.4.3 Invitation and attendance

An administrative database that holds the details of all women included in the target population is needed. The data held should include unique identification for each woman, such as name, date of birth, relevant health or social security numbers, usual doctor (where appropriate), and address for contact.

Population registries can in general provide such data but must be updated regularly to account for population migration, deaths and changes in personal details. In those countries in which population registries are based on administrative areas of small size, communication between registries is essential. Suitable registries might include population, electoral, social security, screening programme, and health service registries.

Cervical smears should not be taken routinely from well women attending contraceptive clinics, ante-natal clinics or post-natal clinics unless the women are over the local age of starting screening and have not had a smear within the previous time period set by the local health authorities. However, it must be emphasised that women with symptoms or signs related to cervical cancer should be investigated at any time.

Women with disabilities should not be excluded from cervical screening because they are at equal risk to the rest of the population. Assessment of the special needs of subsets within the target population, such as ethnic or immigrant minorities with diverse cultural and religious backgrounds is important.

In deciding on local policy the following special groups have to be considered:

- Women who have never been sexually active are at low risk for cervical cancer. However, particularly in young women, this circumstance is subject to change. For practical reasons and to avoid discrimination, all women should be invited for screening irrespective of sexual experience.
- Hysterectomised women can be excluded from screening if surgery was not connected with cervical neoplasia. However, women who have had a sub-total hysterectomy (which leaves the cervix in place) should continue to have cervical screening.

2.4.3.1 How to reach the target population and increase coverage

A fundamental prerequisite for the success of a screening programme is that women in the target population are actually screened. Therefore, special efforts should be made to reach women who were never screened.

Barriers:

The extent to which women participate in screening is associated with age, socio-economic status and marital status. Depending on the local conditions, single women, women from minority ethnic groups, and women of low socio-economic status may be less likely to be screened (Arbyn *et al.*, 1997; IARC, 2005). Often they have never had a smear, and their contact with the health service has not been recent. Personal invitations have been shown to reduce differences in access between such groups (Ronco *et al.*, 1991; IARC, 2005). Non-compliers have higher incidence and mortality risks. Women with disabilities are often excluded from cervical screening, yet many have risk factors equal to the rest of the population. For example, paraplegic women have often been sexually active prior to the trauma that caused their paralysis, and women with various mental handicaps can sometimes combine sexual activity and high rates of smoking.

Fear of gynaecological examination, fear of cancer, social stigma, concern about the gender of the smear taker, non-confidence in the method, discomfort in earlier experiences in the screening services, and in the health care system in general, are all obstacles which are difficult to remove and which are largely dependent on the cultural and social background. The tools for removing them need to be tailored to the individual community to which invitations are addressed. The same is true for potential barriers decreasing accessibility, such as distance from clinics and waiting time. The cost of the test and/or of the consultation fee may be a barrier in some health care systems.

Tools for increasing compliance:

For the population at average risk, general recommendations to achieve high participation rates can be made. It has been shown that individual invitation letters can be very effective. Letters in the name of the woman's own doctor result in higher compliance (Ronco *et al.*, 1997; Segnan *et al.*, 1998). There is compelling evidence that many women experience negative psychological effects from receiving an abnormal smear result. These effects can compromise compliance with subsequent screening and follow-up (IARC, 2005). A more comprehensive guidance on communication with women is presented in Appendix 1.

In the UK, economic incentives for doctors have been shown to be effective in improving coverage, since 1993. Coverage has reached and been maintained at more than 80%, compared with only around 25% in 1988 before target payments and a computerised call and recall system was introduced.

Costs of screening:

In Europe, attending organised screening usually is free of charge (Anttila *et al.*, 2004). Costs for opportunistic screening are covered completely or partially by the health insurance of the individual woman; often a personal contribution is required. If screening is not free or fully covered by insurance, provision should be made for women who are unable to pay. It is important that opportunistic screening is not cheaper for women than the population-based programme.

2.4.4 Screening test and management of screen-positive women

More detailed instructions on how to prepare an adequate Pap smear, how to analyse it, and to assure the best possible quality in a cytological laboratory are presented in Chapters 3 and 4. The following recommendations deal with the programme organisation and data registration and transmission.

2.4.4.1 Smear taking

The request form should be designed to allow easy computer entry of the details of the woman and the smear taker; use of bar-coded labels is encouraged. There should be space on the form to document: the type of sample collected the identification of the woman and the slides, clinical information (such as date of last menstrual period or recent pregnancy) and observations (such as irregular bleeding or suspicious appearance of cervix), screening results and histologically verified findings.

Communication with women is of great importance. The smear taker needs to explain the procedure, telling the woman what to expect and give reassurance. The smear taker should ask the woman about her general health and whether she has any symptoms, such as irregular bleeding or discharge. Any local consent protocol needs to be followed.

Each smear taker and the programme organisation should monitor the frequency of unsatisfactory smears and seek further training, if necessary.

2.4.4.2 Smear interpretation and reporting

Detailed protocols on how to prepare and handle Pap smears must be available and followed (Chapter 3, annex 1). If new tests or modifications to the Pap smear test are to be implemented further information needs to be collected.

Laboratory computers used to generate smear reports should have a system for frequently backing up data in short intervals. If reports are not computer generated, a paper copy of each issued report should be kept for a minimum of ten years.

The laboratory environment and staff training are very important for high quality screening (see Chapters 4 and 5). The cytological result must be classified according to the national standard classification (Chapter 3).

Results can be communicated to women in different ways. In many cases, the woman returns to the smear taker or calls by telephone for information, sometimes a letter is sent, but frequently the woman is not informed if the result is normal. The responsibility for informing women of positive tests must be clear at all times. Ideally, the woman should be informed of her smear result even if it is negative.

2.4.4.3 Management of screen-positive women

The main purpose of a screening test is to classify subjects as likely or unlikely to have the disease that is the object of screening (Morrison, 1992). Following this principle, a clear cut-off value is needed allowing for the binary decision: "test negative" (no further action/return to next screening date) or "further examination required". Usually, the cut-off for referral is set to ASC (corresponding to "borderline dyskariosis" in the UK system or PAP III in the Munich system) or worse, or LSIL or worse. Communication of screening results to women must include a clear operational recommendation (e.g. repeat screening at standard interval, repeat at shorter interval, refer for colposcopy). To permit monitoring, it is also essential that the operational recommendation is registered. Several studies have found a relevant proportion of invasive cancers in women with abnormal cytology which was not managed adequately (Sasieni *et al.*, 1996; Baldauf *et al.*, 1997; Zappa & Ciatto, 2000; Gornall *et al.*, 2000). Therefore it is essential that the screening programme also has a system to find out and remind women who have been referred for diagnosis and/or treatment but have not had the recommended procedure. This is generally referred to as a 'fail safe system'. Frequently women are contacted directly or by telephone if colposcopy is needed. Communication skills are needed in order to reduce anxiety (see also Appendix 1). Providing the address of reference centres and pre-fixed, changeable appointments for colposcopy is expected to increase attendance.

2.4.4.4 Colposcopy and treatment

Screening will not be effective if abnormal smears are not followed adequately and treated if indicated. In order to avoid loss to follow-up, women should have ready access to colposcopy. For high-grade lesions, delay should not be more than 4 weeks. It is not acceptable for cervical lesions to be treated without previous colposcopy. The colposcopy clinic facilities should protect the woman's dignity, and women should be given time to discuss their care prior to, and following the

colposcopy examination, and/or treatment; this should include social support. There should also be suitable access also for those with disabilities.

The cytology result should be available to the colposcopist prior to the colposcopic examination. Ideally, colposcopy services should be audited.

It is essential that the colposcopy result and the advice for future management are clearly explained to the woman. Performed colposcopies (date and patient's identification), histological results, recommended actions and treatment should be registered. This is essential in order to produce the monitoring parameters described in the annex to this chapter.

A fail-safe mechanism helps reminding women who default from follow-up or recommended treatment. Women with symptoms suggestive of or compatible with cervical or other gynaecological cancer – such as unexplained bleeding, a macroscopically visible tumour or ulceration – should have immediate access to diagnostic procedures. Guidelines for management of women with lesions, treatment and follow-up, are discussed in Chapter 6.

2.4.5 Health information systems and registration

A population-based information system is the basic building block of organised screening programmes. The information system should be designed to support the screening programme and enable monitoring and evaluation. It should:

- Identify the target population. For a screening programme, the database incorporates the entire target population
- Identify the individual women in the target population –differentiating unscreened and screened, and women in specially targeted groups
- Permit letters to be sent to the individual women in the target population to:
 - i) Invite or remind to attend for screening when a woman reaches the recommended age, and to re-attend for screening at the recommended interval
 - ii) support early recall, if indicated
- Record the screening findings and identify women for whom further action is recommended.
- Monitor that recommended action has been taken following the detection of an abnormality, and collect information on the further investigations and management
- Provide long-term follow-up for patients who have received treatment
- Identify cancers and deaths in the whole population
- Permit linkage of individual screening episodes, and cancers and pre-cancerous lesions for systematic quality assurance purposes and feed-back to laboratories and clinicians.

Development of information systems will be facilitated by the introduction of permanent individual identifiers. A unique personal identifier should be used, such as national social security number, if available, to avoid person-mismatching. However, establishment of databases to support screening programmes is possible in the absence of unique individual identifiers and should not be delayed if such identifiers are not yet available.

Information system design should not be regarded as a purely technical exercise involving systems experts; the views and data requirements of all groups involved in the screening programme should be considered. A wide range of consultation and participatory planning is essential. Opportunities to improve programme evaluation and delivery may be lost if efforts are not made to coordinate data definitions and standards. It is important that, through appropriate consultation, common definition of data elements be achieved.

2.4.5.1 Registration of the screening programme

Information needs to be collected at the individual level with centralised data collection and reporting systems for the entire programme. Very large target populations require a unified and rapid reporting and administration system for transferring data from regional files to the national statistics; the individual-level registries can be maintained at the regional level and need not be transferred into one central unit. The minimum data recorded for women invited to screening are the personal identifier, the time and place to which she was invited, and the specification whether the invitation was for the regular interval screening or for a follow-up purpose. Socio-demographic information may also be included, as well as information on eligibility. If the screening programme is using a randomised design, for public health policy evaluation (e.g. in routine implementation of new screening technology), the status of the individuals in various randomisation groups need to be included. In a randomised controlled trial this may not be feasible. Files on non-screened or non-invited women in the target population should preferably also be included.

Screening visit files need to include the personal identifier; linkage to the invitational record; screening attendance, including time, place and reason (invitational screening, opportunistic screening, follow-up screening, or testing due to symptoms); clinical information; sample type (conventional or liquid-based cytology); sample quality; the analysing laboratory; screening results and recommendations. The files need also to include confirmatory investigations, including colposcopy, histology, and treatment. This information must contain sufficient detail to complete the data tables presented in the annex to this chapter. Rapid publication of the monitoring tables is important. Since the screening units and various other actors in the field also need the information for running their own activity (e.g. to consult between cytology and histology, to give feedback to the smear-takers, to report on the activity of the unit or laboratory to the screening provider) it is recommended that this data is collected in the field by the screening units or laboratories. Storage and quality control of the data should be performed by a centralised registration unit of the national programme.

Care should be taken to avoid collection of information for which no use is planned. Increasing the administrative workload of screening personnel without appropriate feedback can result in more incomplete and unreliable information.

2.4.5.2 Data collection from opportunistic smears

Opportunistic screening is defined here as the practice of taking smears whenever the opportunity arises (e.g. from women visiting a physician's office for any other purpose) or on women's own initiative. Often, this practice is not monitored because data are not registered. As opportunistic screening exists in most countries, even though opportunistic screening is discouraged, the general recommendation is to include opportunistic screening in the regular screening registration. To do so, full collaboration of all cytology laboratories in the area should be obtained. Each laboratory should transmit in a uniform way computerised data on each smear performed in the catchment area.

Since most of the laboratories handling opportunistic smears are privately operated, with financial constraints, the software needed to transfer data could be financed by the screening organisation. The opportunistic data can be integrated into the screening information system and added to the screening register where all smears of each individual woman should be linked. Monitoring of smear intensity and the number of excess smears can then be performed from the register-based sources. Linking the smear register with the biopsy specimen register and the cancer register will permit evaluation of cytohistology correlation and identification of interval cases. Registration of all smears will also permit monitoring of follow-up outcomes after abnormal cytology and will permit fail-safe measures. Quality assurance will become possible and cytopathologists will be very interested in the outcomes following smears which they have read.

If registration of opportunistic smears is not available, information on opportunistic screening can be collected using questionnaire or interview surveys. When diagnosing a cancer case, information on previous smears, including opportunistic smears should be checked. Re-reading of the previous smears can be performed at this phase to give feedback on potential false-negative result. This activity will serve more an educational rather than an epidemiological purpose, unless controls and blind review of the slides are added. If there is no register-based source, the completeness of such activity cannot be assured.

2.4.5.3 Registration of cervical cancers

Cancer registry data files should be validated as recommended by the European Network for Cancer Registries (Jensen *et al.*, eds, 1991; Parkin *et al.*, eds, 2002; Tyczynski *et al.*, eds, 2003). If a cancer registry does not exist in the screening area, efforts should be made to collect similar information from pathology and hospital files. Cancer registry information should include, as a minimum, the personal identifier, primary site, date and place of diagnosis, histology and stage. CIN3 as well as the micro-invasive carcinomas (FIGO stage Ia) should be recorded separately. Cancer registry files are recommended to be linked also with the causes-of-death files. This improves the information both in the incidence and cause-of-death-files, and also enables calculation of incidence-based refined mortality rates (see glossary). The implementation of screening programmes should not be delayed because of absence of the cancer registry.

2.4.5.4 Storage of biological materials

Information systems also deal with the storage of biological materials, such as archived smears, pre-cancer or cancer tissue blocks, or other tissue samples. The principal use of archived samples by screening programmes is in quality assurance activities such as re-readings or audit. Such infrastructure and materials may also be valuable for other research and evaluation of healthcare services. For a review of the ethical and juridical considerations in bio-banking, we refer to guidelines being developed by the EU-funded CCRPB research project (Cancer Control using Population based Registries and Bio-banking, see: <http://www.cancerbiobank.org/>).

2.4.6 Legal and ethical aspects of data collection and linkage

Confidentiality of information on health status is a fundamental individual right. However, it is also the community that organises screening for healthy participants, and therefore the community has a duty to demonstrate and optimise health benefits and to minimise negative effects and unnecessary cost.

Privacy protection legislation in the member states of the EU complies with the *EU Data Protection Directive 95/46/EC of 24 October 1995 of the European Parliament and of the Council on the protection of natural persons with regard to the processing of personal data and on free movement of such data*. In principle, registration of personal medical data without informed consent of the data subject concerned is prohibited. National legislation can provide derogations, however, that allow processing of such data by health professionals subject to professional secrecy, in the framework of preventive or curative care to patients, management of health services, and scientific research (Arbyn *et al.*, 1999). Information of the data subject is obligatory when personal data are transmitted to a third party. This obligation can be waived, if providing the information involves excessive efforts. When implementing screening programmes, national privacy legislation should be checked as to whether derogation of the obligation of informed consent is foreseen in the frame-

work of cancer registration. Depending on the national implementation of the EU data protection directive, data can also be used for research purposes (such as evaluation of screening) without patient consent.

The European Network of Cancer Registries (ENCR) has studied the consequences of the EU Data Protection Directive on the registration of newly diagnosed cancer cases and has developed guidelines (Storm *et al.*, 2004). These guidelines should be applied in collection, processing, storage and release of data on cancer screening. The Council Recommendation on cancer screening emphasises this principle (Council of the European Union, 2003; European Commission, 2003).

Whenever possible, the same principles should be applied to organised and opportunistic screening. Local agreements with data providers (e.g., laboratories for cytopathology and physicians) will have to be established, stipulating all ethical aspects of data transfer and security. Registration of all Pap smear results and subsequent histological information from all individuals, independent of the reasons for testing (organised or opportunistic) may have practical advantages for cytopathology laboratories.

The screening register should contain individual screening test and follow-up histories, and should be linkable to population registers (allowing invitation of women from the target population), and to the cancer registry (in order to identify interval cancers). Cancer registries in turn, should be linkable to mortality registers that allow completion of cancer registration and evaluation of survival of diagnosed cancer patients (Muir & Démaret, 1991). Persons responsible for the organisation and evaluation of screening should assure that these linkages are legally possible and, if not, propose adaptation of legislation. Adequate safeguards should be applied as laid down in national law or in local administrative rules.

Completeness, accurateness and reliability of data collection and processing are important quality issues. To avoid person mismatching, a unique personal identifier such as national register number or social security number should be used, if available. Auditing of the achievement of the programme objectives should be considered as an ethical requirement that distinguishes "population screening" from "opportunistic" screening on the individual initiative of a patient or her physician (Sasieni & Cuzick, 2001).

In conclusion, implementation of well-designed and monitored information systems can enhance the benefits of an organised, nationwide screening programme. Effective information systems can help to ensure quality control by linking testing and treatment with outcomes; they may also be used to increase efficiency, decrease harmful effects, identify under-screening of risk groups (e.g., elderly women), support programme evaluation, and answer research questions. These results can be fed back and used for further programme improvement.

2.5 Monitoring and evaluation

2.5.1 Screening outcome

The programme design should permit evaluation. One can distinguish between screening as a research exercise and screening as a public health policy. The outcomes of both can be evaluated using a randomised design.

The purpose of screening for cancer is to reduce disease-specific mortality. Therefore, the primary indicator of effect is the observed mortality compared with the expected mortality in the absence of screening. For cervical cancer, the pre-invasive disease is detected by screening and therefore reduction in incidence of fully invasive cancer is also a valid indicator of effectiveness, in which case the condition being prevented by screening is future deaths (IARC, 2005). Invasive cancer rates may also be compared with those of high-grade CIN, particularly CIN3 in the same population.

In addition to favourable effects, evaluation should also consider unfavourable effects, see below. Adverse outcomes need to be included and balanced against the advantages in the evaluation of a screening programme.

Process and intermediate indicators (see section 2.5.2) are often used in the evaluation of screening. An assessment of the screening programme based on process indicators alone has limitations, because ineffective programmes may also show some favourable changes in process indicators. Therefore, evaluation should also include outcome indicators. The 'true' disease state being sought at the time of screening is a lesion that will progress into an invasive cancer (IARC, 2005). Due to treatment, progression of screen-detected lesions is not directly observable. However, the invasive cervical cancer cases prevented by screening can be estimated by comparing subsequent invasive cervical cancer incidence among screened populations to that expected in the absence of screening. A similar approach can also be taken to specificity and positive predictive value of the screening test and the screening episode. It is of a special interest to estimate the proportion of lesions detected at screening that would have progressed to clinical cancers before the next screen (*ibid.*; for regression/progression probabilities and length of pre-clinically detectable phase see also section 2.3.2). This is a perspective for addressing issues related to potential over-diagnosis or over-treatment.

Non-experimental outcome evaluation:

If an appropriate unscreened population is available for direct comparison, as in a randomised trial, the quantity of cervical cancers that were prevented is directly observable (IARC, 2005). In the absence of a strictly defined, randomised comparison group, estimates based on age-adjusted cancer incidence or mortality data from a comparable population or a time when screening was not practised should provide an approximation if used judiciously. As mentioned earlier, no randomised studies on the efficacy of cervical cancer screening are available. If effects are large, and if no other factors can explain such changes, they may reasonably be accepted as evidence of the effectiveness of screening.

Few cohort and case-control studies have been conducted to evaluate screening programmes (IARC, 2005). Instead, most data on the effectiveness of screening stems from time trends and geographical differences between populations subjected to screening of variable intensity (section 2.3.4). Cohort studies involve a follow-up comparison between the screened target population and a relevant control population. The results of a cohort study are given in terms of absolute rates and relative risks. A cohort study requires that individuals in the target and control populations can be identified and followed. Follow-up involves linkage of the screening data with data on subsequent disease.

Comparison of the outcome in screened versus non- or less-screened populations using a cohort design potentially suffers from a series of selection and confounding factors. There might be differences in levels or trends of background risk, or differences in health care systems between geographical areas. Screened and non-screened women may differ with respect to several risk factors, health status or general health behaviour. Estimating effects among all invited persons (or those otherwise offered screening) or in the total target population is therefore the preferred method. When participation in screening is not randomised, differences in incidence cannot be attributed entirely to screening. However, methods are available to correct for selective attendance (Cuzick *et*

al., 1997; see IARC 2005 for a more detailed description on the methodology and biases in cohort follow-up studies).

Experimental evaluation of outcome:

The effect of cytological screening compared with no screening on the risk of invasive cervical cancer can be large, depending on the quality of cytology. Screening with a new test is likely to have considerably smaller additional effect on incidence. Assessment of such a small effect requires large sample sizes and follow-up over several years.

The classical randomised trial, aiming to show increased reduction in cancer occurrence in the experimental compared to the control group, might be considered as too expensive and impractical. An acceptable and feasible alternative may be an implementation policy involving randomised screening. The control group (receiving the standard screening test) and experimental groups(s) receiving the new test are made up of individuals randomised from the target population. The randomised screening policy should start before a new method has penetrated into routine practice or is used for spontaneous screening. Otherwise, clinicians or women who prefer to use the new method might create ethical problems and result in protocol contamination. It is ethically acceptable to carry out a randomised screening policy for evaluation, if resources are limited and the new technique can only be offered to a proportion of the population, and provided that the new test is withheld from no one in the experimental group and the trial gives an a priori equal chance to those in the target population to benefit or avoid any adverse effects from the new test.

The randomised screening policy can be applied in settings with high quality standards of organisation, in which the established monitoring and evaluation systems can be used to assess outcomes. In such a situation, limited additional resources can be sufficient to run the evaluation. Countries with well-organised screening programmes offer excellent settings for evaluation of a new technology.

The Finnish programme provides an example of a randomised public health policy. Cervical cancer is very rare in women after negative screening cytology: the cumulative incidence is 0.03% for a follow-up time of one screening interval (5 years) (Vikki *et al.*, 1999). Given the small proportion of screening-detected lesions evaluation of the impact of screening on cancer incidence is a priority. A 2:1 randomised, prospective trial on automation-assisted screening is being carried out as a part of the national screening programme for cervical cancer (Nieminen *et al.*, 2005). Using the national population registry, a large number of women (ca. 500,000), aged 30–60 (25–65 in some municipalities) have been invited since 1999. These women were randomised individually into 2 arms to have their smear analysed either conventionally (2/3) or with the automation-assisted method (1/3), within the organised screening programme. Randomisation was performed by national authorities based on random allocation using the personal identification number issued to every resident in Finland. The results available to date demonstrate the feasibility and acceptability of the study design and confirm the need for a design that allows identification of even small effects on performance indicators.

The Finnish study has been expanded to a multi-arm design, with introduction of an HPV-DNA screening arm in 2003. Other randomised screening trials of HPV-DNA screening, following mainly the concept of a clinical trial, are currently underway in the Italian, Dutch, UK, and Swedish cervical screening programmes (Davies *et al.*, 2006)

In addition to the overriding aim of demonstrating effectiveness, it is also important to study in detail any additional information resulting from screening tests and related investigations. There is large variability in the diagnostic criteria of precursors. Therefore, research is needed to validate the potential of intermediate outcomes to further reduce cancer incidence and mortality. As mentioned above, cross-sectional test performance does not provide sufficient evidence for the effectiveness of a new screening technique. This information is important, however, in deciding when

prospective evaluation of a new technique in the routine programme is warranted. The principles of evaluation of new screening methods are covered in more detail in Chapter 3.

Introduction of emerging screening technologies based on insufficiently epidemiologically validated outcomes can have serious drawbacks: increasing costs, potential over-diagnosis and over-treatment, spurious expectation of increased efficacy and delayed establishment of evidence. The recommended experimental design is an important tool aiming to overcome these consequences.

A randomised screening design can also be used to demonstrate the impact of other alternative policies. For example, different methods of invitation or different target age groups (i.e., different ages for starting or stopping screening) can be compared; and tools for improving equity or organising quality assurance can be studied. Depending on the issue and screening organisation, a cluster randomisation method may sometimes be suitable. A randomised screening design may also be helpful in the build-up phase of a programme: e.g., if resources are not yet available for the whole target population, if all the healthcare services and other required infrastructure have not yet been evaluated, and if there is no certainty that the desired outcome and quality will be reached in that particular programme.

Evaluation of adverse effects of screening:

Screening benefit is counteracted by the adverse effects of testing large populations of predominantly healthy women to prevent significant disease in a few (see IARC 2005, pp 214). Such effects include misunderstanding of the meaning of positive test results by women and health care providers (interpretation as 'cancer'), psychological consequences of positive test results (increased anxiety and fear), misunderstanding of the meaning of negative test results by women and health care providers (interpretation as 'no risk' rather than 'low risk' implying potential of under-investigation of symptoms), false-positive test results leading to unnecessary referrals implying additional psychological and financial costs, false-negative results implying the potential of delayed intervention against symptomatic disease occurring within the screening interval, over-diagnosis and over-treatment of pre-invasive lesions that would never have progressed to clinically significant disease entailing the risk of treatment complications (cervical stenosis, cervical incompetence, potential adverse effects on reproductive health).

Only a few empirical investigations have been reported on adverse effects of cervical screening, but the available data underline the relevance of the subject and the need for further research. The risk of over-diagnosis and over-treatment of otherwise clinically insignificant lesions results not only from the fact that there may be false positive abnormal smears (Insinga *et al.*, 2004); the studies summarised in section 2.3.2 also indicate the low progression rate of CIN 1 and CIN2 lesions. Van Ballegooijen *et al.* showed in a model-based analysis of the Dutch programme that an increasing number of screening tests in a fixed population implies an increasing number of referrals and an increasing number of minor treatment procedures with a decrease in prevented cervical cancer deaths (van Ballegooijen *et al.*, 1990). In a recent review of the literature it was also concluded that excisional treatment of CIN is associated with a significantly increased risk of preterm delivery and low birth weight (Kyrgiou *et al.*, 2006).

2.5.2 Monitoring

Screening is a complex activity including different steps. Monitoring is the process of continuous, ongoing evaluation to determine the quality of these steps and whether a programme is achieving intermediate objectives. For this purpose, "process measures" are used. Of themselves these process measures are not indicators of the success of a screening programme. If comprehensive in

scope they indicate, however, whether or not the programme is proceeding in a manner likely to achieve successful results, because such results are unlikely if performance targets are not met.

The final objective of cervical screening is to reduce the incidence and mortality from cervical cancer, with the lowest burden and least adverse effects for women (human costs) and at the lowest economic cost. Monitoring provides early feedback in order to identify problems and to make necessary changes. Continuous and comprehensive monitoring systems that cover both organised and opportunistic screening are required, particularly because opportunistic activity is widespread in many European countries. It is recognized that difficulties in obtaining data from opportunistic activity may be expected, especially when a large number of health services and professionals are involved in such activity.

For instance, the proportion of women screened depends, among other things, on the proportion of women actually subject to active invitation and on the compliance with such invitation. It is essential to limit the proportion of undelivered invitations, i.e., to maintain the quality of invitation lists. The delivery of diagnostic work-up and treatment also depends on effective communication of results.

Annex 1 of this chapter presents standard tables that can be used for reporting the main characteristics of screening programmes and for computation of the performance indicators, which are discussed in Chapter 7. These tables should be considered as a template for standardised monitoring of screening performance in the EU. Each member state should be able to fill in these or similar tables and make data available for inter-country comparison of basic performance indicators, in order to promote exchange of experience in best practice as recommended by the Council of the European Union (2003).

The current recommendation is that statistical reports should be produced and published at regular intervals, for a screening round of 3 or 5 years as well as annually. Use of longer periods than a screening round are also recommended for the monitoring activity. Because regular evaluation and monitoring data including all screening tests and subsequent actions in the screening programmes are not available, common European benchmarks for monitoring the performance of the programmes are not yet recommended. Such benchmarks could be considered in the future after receiving monitoring data as well as comparative information on the biological background risk and long-term effectiveness of the programmes. Meanwhile, national benchmarks can be considered as an option for the member countries.

The tables and parameters are meant to cover the entire screening activity, but some of them can also be applied to single structures (taking into account problems resulting from small size). For example, specificity or positive predictive value (PPV) can be computed by the laboratory that reported the smear, or even by the screener. It must be kept in mind that, in order to provide correct measures, data need to be collected at a population level. Thus, PPV can be correctly computed only when considering all biopsies related to the studied cytological tests, including those interpreted in different units. The relevance of this problem depends on local conditions.

Currently, separate monitoring systems are implemented at a national or regional level in several member states. In other member states, information systems are lacking. This makes comparison between different areas in Europe difficult and is one reason for the standardised parameters recommended in the present guideline.

Given the shortcomings of information systems in many countries, only some of the recommended tables and some of the detailed data will be currently available. An effort to change existing information systems is expected. Given the variability between European countries, perfect standardisation and detailed instructions fully applicable in all countries is currently not feasible. An effort to validate the current guideline tables in different European countries is therefore needed.

Data originally registered for other reasons can be routinely integrated into the screening information system. In the absence of routinely produced population-based data, periodic ad-hoc surveys can be considered. For example, estimates of coverage in France were obtained by analysis of insurance data (Rousseau *et al.*, 2002; Arbyn & Van Oyen, 2004). Interview surveys have been used to estimate coverage in various European countries (Arbyn *et al.*, 1997; Kahl *et al.*, 1999; AETS, 2002; Arbyn & Van Oyen, 2004; Mancini *et al.*, 2004). However, a tendency to overestimate attendance by interview was observed in many studies (Walter *et al.*, 1988; Montano & Phillips, 1995; Arbyn & Van Oyen, 2004).

2.5.3 Auditing screening histories of cancer cases

When screening data can be linked with the cancer registry, a comprehensive evaluation with a systematic audit process of the entire screening programme can be performed. Each case of cancer should be investigated, i.e., cancers in both screened and unscreened women. Whenever possible, screen-detected cancers should be distinguished from symptomatic cancers. As the evolution from a pre-cancerous lesion to invasive disease usually requires much more than one screening round, the review should include not only the interval cancers but also invasive cancers that are diagnosed at subsequent screens. CIN3 cases, if detected between screens, may also be used in an audit.

In cancers diagnosed in unscreened women, invitation and compliance with invitation should be examined. A systematic audit will distinguish between failures in invitation and failures in compliance with invitation. Feedback on this issue is particularly instructive for the persons in charge of organising screening.

Review of negative cytological slides of subsequent cases, seeded in a relevant set of control slides and including both blinded and non-blinded assessments, will allow distinction between: errors in cytological interpretation (obvious human errors or slides containing very few abnormalities), problems attributable to sample quality, and difficulties in development of diagnostic criteria. This re-reading should involve both the original and an external (reference) laboratory. Registry-based audit should be carried out for any screening technologies that are implemented in the programme. To study specificity criteria, samples of false positive tests can also be included in an audit. Systematic audits should also examine colposcopy management, histological diagnosis, and adequacy of treatment and follow-up of pre-cancerous lesions. Audits should also assess compliance with recommendations for repeat smears and colposcopy and should determine whether those recommendations were appropriate.

Cases should be discussed in a multi-disciplinary forum so that factors that resulted in cancers not being prevented can be put in the context of other factors, stage of cancer and whether or not the cancer was screen-detected (screen-detected cancers also include those detected in a follow-up process).

Feedback of the results of such systematic audits to the concerned health professionals is very instructive, but must be done with caution, respecting local rules. An important element of the audit is to follow and monitor the laboratories over the long term, to demonstrate whether the quality assurance activity contributed to any additional effectiveness, and to identify key barriers. It is important to verify whether the sensitivity improved without losing a good level of specificity, and to monitor the treatment rates to check for over-diagnosis. Checking and improving register data quality is also a task within the audit.

Chapters 4, 5 and 6 describe the auditing activities in the cytological laboratory and for the diagnostic and therapeutic management of screen-positive subjects.

2.5.4 Cost-effectiveness

Prior to the decision to initiate or change a screening programme, cost-effectiveness analyses should be carried out. To be comprehensive, the cost for the health system of each step of the programme and screening policy options should be evaluated: invitations and attendance; smear taking; modifications of the screening test systems; re-testing and follow-up procedures; management strategies; and documentation, registration, monitoring and evaluation. After deciding on the national screening policy or programme, based on the prior cost-effectiveness assessments, it is recommendable to implement any large changes in the policy or laboratory systems, or start a new programme in a step-wise manner: e.g. to pilot first, in order to assess feasibility, and demonstrate that effectiveness and costs of the programme or policy were at the expected level.

Invasive and pre-invasive lesions have various screen-detectable or pre-clinical states. By generating individual life histories, a dynamic population can be simulated that represents the demography, mortality of all causes, and the incidence and mortality from cervix cancer. In the disease part of such a programme, the relevant stages of cervical cancer are distinguished and the natural history is simulated as a progression through the stages. Key parameters in modelling the performance of screening are the mean duration of screen-detectable pre-clinical disease, sensitivity, and improvement of prognosis for screen-detected cancers. Computer simulation packages such as MISCAN, developed by the Erasmus University in Rotterdam (The Netherlands), and other modelling techniques based on Markov and Monte Carlo computer models have been employed in cost-effectiveness analysis (van Ballegooijen *et al.*, 1992; van Ballegooijen *et al.*, 2000; van den Akker van Marle *et al.*, 2002; Goldie, 2002; Sherlaw-Johnson & Philips, 2004; Salomon *et al.*, 2004).

In the short term, the prerequisites and costs of screening activities vary substantially between programmes, and also between screening technologies, depending on the requisite re-organisation of activities and re-distribution of resources within existing health-care systems. Differences in sample taking, in sample processing and analysis (traditional vs. liquid-based smear vs. HPV test), in volume and distribution of screening tests, excess consumption of tests and/or treatment, and cost per analysed test are examples. In the long term, the results of the cost-effectiveness analyses depend greatly upon the observed effectiveness of the programme (Hristova & Hakama, 1997).

2.6 References

Advisory Committee on Cancer Prevention & Lynge E. (2000). Recommendations on cancer screening in the European Union. Advisory Committee on Cancer Prevention. *Eur J Cancer* **36**: 1473-1478.

AETS (2002). Uso de la mamografía y de la citología de Papanicolau para la detección precoz del cáncer de mama y de cervix uterino en España. Agencia de Evaluación de Tecnologías Sanitarias del Instituto de Salud Carlos III, Madrid.

- Anttila A. & Laara E. (2000). Cervix cancer: Geographical correlations. In: *Evaluation and Monitoring of Screening Programmes* (eds Sankila R., Demaret E., Hakama M., Lynge E., Schouten L.J. & Parkin D.M.), pp. 77-97. Europe Against Cancer Programme, Brussels, Luxembourg.
- Anttila A. & Nieminen P. (2000). Cervical cancer screening programme in Finland. *Eur J Cancer* **36**: 2209-2214.
- Anttila A., Pukkala E., Soderman B., Kallio M., Nieminen P., & Hakama M. (1999). Effect of organised screening on cervical cancer incidence and mortality in Finland, 1963-1995: recent increase in cervical cancer incidence. *Int. J. Cancer* **83**: 59-65.
- Anttila A., Ronco G., Clifford G., Bray F., Hakama M., Arbyn M., & Weiderpass E. (2004). Cervical cancer screening programmes and policies in 18 European countries. *Br. J. Cancer* **91**: 935-941.
- Arbyn M., Autier P., & Ferlay J. (2007b). Burden of cervical cancer in the 27 member states of the European Union: estimates for 2004. *Ann. Oncol.* **18**: 1425-1427. (2007b).
- Arbyn M. & Geys H. (2002). Trend of cervical cancer mortality in Belgium (1954-94): tentative solution for the certification problem of not specified uterine cancer. *Int. J. Cancer* **102**: 649-654.
- Arbyn M., Quataert P., Van Hal G., & Van Oyen H. (1997). Cervical cancer screening in the Flemish Region (Belgium): measurement of the attendance rate by telephone interview. *Eur J Cancer Prev* **6**: 389-398.
- Arbyn M., Raifu A.O., Autier P., & Ferlay J. (2007a). Burden of cervical cancer in Europe: estimates for 2004. *Ann. Oncol.* **18**: in press-doi: 10.1093/annonc/mdm079. (2007a).
- Arbyn M. & Van Oyen H. Analysis of individual health insurance data pertaining to Pap smears, colposcopies, biopsies and surgery on the uterine cervix (Belgium, 1996-2000). IPH/EPI-REPORTS 21, 1-100. 2004. Brussels, Scientific Institute of Public Health
- Arbyn M., Wallyn S., Van Oyen H., Nys H., Dhont J., & Seutin B. (1999). The new privacy law in Belgium: a legal basis for organised cancer screening. *Eur J Health Law* **6**: 401-407.
- Baldauf J.J., Dreyfus M., Ritter J., Meyer P., & Philippe E. (1997). Screening histories of incidence cases of cervical cancer and high grade SIL. A comparison. *Acta Cytol.* **41**: 1431-1438.
- Berrino F. (2003). The EURO CARE Study: strengths, limitations and perspectives of population-based, comparative survival studies. *Ann. Oncol.* **14 Suppl 5**: v9-13.
- Berrino F., Gatta G., d'Alto M., Crosignani P. & Riboli E. (1984). Use of case-control studies in evaluation of screening programmes. In: *Screening for cancer I: general principles on evaluation of screening for cancer and screening for lung, bladder and oral cancer*. (eds Prorok P.C. & Miller A.B.), pp. 29-x. UICC, Geneva.
- Boyes D.A., Morrison B., Knox E.G., Draper G.J., & Miller A.B. (1982). A cohort study of cervical cancer screening in British Columbia. *Clin Invest Med* **5**: 1-29.
- Bray F., Loos A.H., McCarron P., Weiderpass E., Arbyn M., Moller H., Hakama M., & Parkin D.M. (2005). Trends in cervical squamous cell carcinoma incidence in 13 European countries: changing risk and the effects of screening. *Cancer Epidemiol. Biomarkers Prev.* **14**: 677-686.
- Bray F., Sankila R., Ferlay J., & Parkin D.M. (2002). Estimates of cancer incidence and mortality in Europe in 1995. *Eur J Cancer* **38**: 99-166.

Clarke E.A. & Anderson T.W. (1979). Does screening by "Pap" smears help prevent cervical cancer? A case-control study. *Lancet* **2**: 1-4.

Coleman D., Day N., Douglas G., Farmery E., Lynge E., Philip J., & Segnan N. (1993). European Guidelines for Quality Assurance in Cervical Cancer Screening. Europe against cancer programme. *Eur J Cancer* **29A Suppl 4**: S1-S38.

Comber H. & Gavin A. (2004). Recent trends in cervical cancer mortality in Britain and Ireland: the case for population-based cervical cancer screening. *Br. J. Cancer* **91**: 1902-1904.

Council of the European Union (2003). Council Recommendation of 2 December 2003 on cancer screening (2003/878/EC). *Off. J. Eur. Union* L 327/34-38.

Davies P., Arbyn M., Dillner J., Kitchener H., Ronco G., & Hakama M. (2006). A report on the current status of European research on the use of human papillomavirus testing for primary cervical cancer screening. *Int. J. Cancer* **118**: 791-796.

Dickman P.W., Hakulinen T., Luostarinen T., Pukkala E., Sankila R., Soderman B., & Teppo L. (1999). Survival of cancer patients in Finland 1955-1994. *Acta Oncol.* **38 Suppl. 12**: 1-103.

Dillner J. (2000). Cervical cancer screening in Sweden. *Eur J Cancer* **36**: 2255-2259.

Engeland A., Haldorsen T., Tretli S., Hakulinen T., Hørte L.G., Luostarinen T., Magnus K., Schou G., Sigvaldason H., Storm H.H., Tulinius H., & Vaitinen P. (1993). Prediction of cancer incidence in the Nordic countries up to the years 2000 and 2010. A collaborative study of the Five Nordic Cancer Registries. *APMIS Suppl* **38**: 1-124.

European Commission (2003). Proposal for a Council Recommendation on Cancer Screening (presented by the Commission). Brussels, 5.5.2003. COM(2003) 230 final. 2003/0093 (CNS), 1-21.

Goldie S.J. (2002). Health economics and cervical cancer prevention: a global perspective. *Virus Res.* **89**: 301-309.

Gornall R.J., Boyd I.E., Manolitsas T., & Herbert A. (2000). Interval cervical cancer following treatment for cervical intraepithelial neoplasia. *Int. J. Gynecol. Cancer* **10**: 198-202.

Hakama M. (1982). Trends in the incidence of cervical cancer in the Nordic countries. In: *Trends in Cancer Incidence* (ed Magnus K.), pp. 279-292. Hemisphere Publishing Corporation, Washington.

Hakama M., Miller A.B., & Day N.E. (1986). Screening for cancer of the uterine cervix. From the IARC Working Group on Cervical Cancer Screening and the UICC Project Group on the Evaluation of Screening Programmes for Cancer. *IARC Sci. Publ.* , 1-315.

Hakama M. & Räsänen-Virtanen U. (1976). Effect of a mass screening program on the risk of cervical cancer. *Am. J. Epidemiol.* **103**: 512-517.

Hristova L. & Hakama M. (1997). Effect of screening for cancer in the Nordic countries on deaths, cost and quality of life up to the year 2017. *Acta Oncol.* **36 Suppl 9**: 1-60.

IARC (2005). Cervix Cancer Screening. IARC Handbooks of Cancer Prevention. Vol. 10. IARCPress, Lyon

Insinga R.P., Glass A.G., & Brenda B.R. (2004). Diagnoses and outcomes in cervical cancer screening: a population-based study. *Am. J. Obstet. Gynecol.* **191**: 105-113.

Jensen O.M. & Storm H.H.(1991). Cancer registration: principles and methods. IARC Sci. Publ. **(95)**.

Johannesson G., Geirsson G., Day N., & Tulinius H. (1982). Screening for cancer of the uterine cervix in Iceland 1965--1978. *Acta Obstet Gynecol Scand* **61**: 199-203.

Kahl H., Hölling H., & Kamtsiuris P. (1999). Utilization of health screening studies and measures for health promotion. *Gesundheitswesen* **61 Suppl**: 163-168.

Kyrgiou M., Koliopoulos G., Martin-Hirsch P., Arbyn M., Prendiville W., & Paraskevaidis E. (2006). Obstetric outcomes after conservative treatment for intra-epithelial or early invasive cervical lesions: a systematic review and meta-analysis of the literature. *Lancet* **367**: 489-498.

Laara E., Day N.E., & Hakama M. (1987). Trends in mortality from cervical cancer in the Nordic countries: association with organised screening programmes. *Lancet* **1**: 1247-1249.

Linos A. & Riza E. (2000). Comparisons of cervical cancer screening programmes in the European Union. *Eur J Cancer* **36**: 2260-2265.

Luthra U.K., Prabhakar A.K., Seth P., Agarwal S.S., Murthy N.S., Bhatnagar P., Das D.K., & Sharma B.K. (1987). Natural history of precancerous and early cancerous lesions of the uterine cervix. *Acta Cytol.* **31**: 226-234.

Lynge E. (2000). Cohort studies in evaluation of cervical cancer screening. In: *Evaluation and Monitoring of Screening Programmes* (eds Sankila R., Démaret E., Hakama M., Lynge E., Schouten L.J. & Parkin D.M.), pp. 119-131. Europe Against Cancer Programme, Brussels, Luxemburg.

Lynge E., Madsen M., & Engholm G. (1989). Effect of organized screening on incidence and mortality of cervical cancer in Denmark. *Cancer Res.* **49**: 2157-2160.

Magnus K., Langmark F., & Andersen A. (1987). Mass screening for cervical cancer in Ostfold county of Norway 1959-77. *Int. J. Cancer* **39**: 311-316.

Mancini E., Segnan N. & Ronco G. (2004). I determinanti del ricorso allo screening dei tumori femminili. Rome

Miller A.B. (1992). Cervical Cancer Screening Programmes. Managerial Guidelines. World Health Organization, Geneva.

Miller A.B. (2002). The (in)efficiency of cervical screening in Europe. *Eur J Cancer* **38**: 321-326.

Miller A.B., Lindsay J., & Hill G.B. (1976). Mortality from cancer of the uterus in Canada and its relationship to screening for cancer of the cervix. *Int. J. Cancer* **17**: 602-612.

Montano D.E. & Phillips W.R. (1995). Cancer screening by primary care physicians: a comparison of rates obtained from physician self-report, patient survey, and chart audit. *Am. J. Public Health* **85**: 795-800.

Muir C.S. & Démaret E. (1991). Cancer registration : legal aspects and confidentiality. In: *Cancer Registration. Principles and methods.* (eds Sensen O.M., Parkin D.M., MacLennan R., Muir C.S. & Skeet R.G.), pp. 199-207. IARC Sci. Publ. **95**, Lyon.

Nieminen P., Kallio M., Anttila A., & Hakama M. (1999). Organised vs. spontaneous Pap-smear screening for cervical cancer: A case-control study. *Int. J. Cancer* **83**: 55-58.

Nieminen P., Kotaniemi L., Hakama M., Tarkkanen J., Martikainen J., Toivonen T., Ikkala J., Luostarinen T., & Anttila A. (2005). A randomised public-health trial on automation-assisted screening for cervical cancer in Finland: performance with 470,000 invitations. *Int. J. Cancer* **115**: 307-311.

Nygard J.F., Skare G.B., & Thoresen S.O. (2002). The cervical cancer screening programme in Norway, 1992-2000: changes in Pap smear coverage and incidence of cervical cancer. *J Med Screen.* **9**: 86-91.

Ostor A.G. (1993). Natural history of cervical intraepithelial neoplasia: a critical review. *Int. J. Gynecol. Pathol.* **12**: 186-192.

Parkin DM, Bray F, Ferlay J, Pisani P. (2005) Global cancer statistics 2002. *CA Cancer J. Clin.* 55:74-108.

Parkin DM, Whelan SL, Ferlay J, Teppo L, Thomas DB, eds. (2002) Cancer Incidence in Five Continents. IARC Sci. Publ. No. 155, Vol. VIII. Lyon. IARC Press

Patnick J. (2000). Cervical cancer screening in England. *Eur J Cancer* **36**: 2205-2208.

Peto J., Gilham C., Fletcher O., & Matthews F.E. (2004). The cervical cancer epidemic that screening has prevented in the UK. *Lancet* **364**: 249-256.

Quinn M., Babb P., & Jones J. (1999). Effect of Screening on Incidence and Mortality from Cancer of the Cervix in England: Evaluation Based on Routinely Collected Statistics. *BMJ* **318**: 904-908.

Ronco G., Pilutti S., Patriarca S., Montanari G., Ghiringhello B., Volante R., Giordano L., Zanetti R., Mancini E., & Segnan N. (2005). Impact of the introduction of organised screening for cervical cancer in Turin, Italy: cancer incidence by screening history 1992-98. *Br. J. Cancer* **93**: 376-378.

Ronco G., Segnan N., Giordano L., Pilutti S., Senore C., Ponti A., & Volante R. (1997). Interaction of spontaneous and organised screening for cervical cancer in Turin, Italy. *Eur J Cancer* **33**: 1-6.

Ronco G., Segnan N., & Ponti A. (1991). Who has Pap tests? Variables associated with the use of Pap tests in absence of screening programmes. *Int J Epidemiol* **20**: 349-353.

Rousseau A., Bohet P., Merlišre J., Treppoz H., Heules-Bernin B., & Ancelle-Park R. (2002). Evaluation du dépistage organisé, et du dépistage individuel du cancer du col de l'utérus: utilité, des données de l'Assurance maladie. *Bull. Epidemiol. Hebdom.* **19**: 81-84.

Salomon J.A., Weinstein M.C., & Goldie S.J. (2004). Taking account of future technology in cost effectiveness analysis. *BMJ* **329**: 733-736.

Sankila R., Demaret E., Hakama M., Lynge E., Schouten L.J., & Parkin D.M. (2000). Evaluation and monitoring of screening programmes, Office for Official Publications of the European Communities edn.

Sant M., Aareleid T., Berrino F., Bielska L.M., Carli P.M., Faivre J., Grosclaude P., Hedelin G., Matsuda T., Moller H., Moller T., Verdecchia A., Capocaccia R., Gatta G., Micheli A., Santaquilani M., Roazzi P., & Lisi D. (2003). EUROCARE-3: survival of cancer patients diagnosed 1990-94-results and commentary. *Ann.Oncol.* **14 Suppl. 5**: V61-V118.

Sasieni P. & Adams J. (1999). Effect of screening on cervical cancer mortality in England and Wales: analysis of trends with an age period cohort model. *BMJ* **318**: 1244-1245.

Sasieni P., Adams J., & Cuzick J. (2003). Benefit of cervical screening at different ages: evidence from the UK audit of screening histories. *Br. J. Cancer* **89**: 88-93.

Sasieni P. & Cuzick J. (2001). Routine audit is an ethical requirement of screening. *BMJ* **322**: 1179.

Sasieni P., Cuzick J., & Farmery E. (1995). Accelerated decline in cervical cancer mortality in England and Wales. *Lancet* **346**: 1566-1567.

Sasieni P.D., Cuzick J., Lynch-Farmery E.L., & National Co-ordinating Network for Cervical Screening Working Group (1996). Estimating the efficacy of screening by auditing smear histories of women with and without cervical cancer. *Br. J. Cancer* **73**: 1001-1005.

Schaffer P., Sancho-Garnier H., Fender M., Dellenbach P., Carbillet J.P., Monnet E., Gauthier G.P., & Garnier A. (2000). Cervical cancer screening in France. *Eur J Cancer* **22**: 2215-2220.

Segnan N., Ronco G., & Ciatto S. (2000). Cervical cancer screening in Italy. *Eur J Cancer* **36**: 2235-2239.

Segnan N., Senore C., Giordano L., Ponti A., & Ronco G. (1998). Promoting participation in a population screening program for breast and cervical cancer: a randomized trial of different invitation strategies. *Tumori* **84**: 348-353.

Sherlaw-Johnson C. & Philips Z. (2004). An evaluation of liquid-based cytology and human papillomavirus testing within the UK cervical cancer screening programme. *Br. J. Cancer* **91**: 84-91.

Sigurdsson K. (1995). Quality assurance in cervical cancer screening: The Icelandic experience 1964-1993. *Eur J Cancer* **31 A**: 728-734.

Storm H., Buiatti E., Hakulinen T., & Ziegler H. (2004). Guidelines on confidentiality in population-based cancer registration in the European Union. Lyon 1-20.

Syrjänen K.J. (1996). Spontaneous evolution of intraepithelial lesions according to the grade and type of the implicated human papillomavirus (HPV). *Eur. J. Obstet. Gynecol. Reprod. Biol.* **65**: 45-53.

Tyczynski, J.E., Démaret, E., and Parkin, D.M. (2003) Standards and Guidelines for Cancer Registration in Europe. IARC Technical Publications, no.40. Lyon: IARCPress.

Verdecchia A, Francisci S, Brenner H, Gatta G, Micheli A, Mangone L, Kunkler I, and the EURO CARE-4 Working Group. Recent cancer survival in Europe: a 2000-02 period analysis of EURO CARE-4 data. *Lancet Oncology*; published on-line August 21, 2007 (DOI:10.1016/S1470-2045(07)70246-2).

van Ballegooijen M., Habbema J.D., van Oortmarssen G.J., Koopmanschap M.A., Lubbe J.T., & van Agt H.M. (1992). Preventive Pap-smears: balancing costs, risks and benefits. *Br. J. Cancer* **65**: 930-933.

van Ballegooijen M. & Hermens R. (2000). Cervical cancer screening in the Netherlands. *Eur J Cancer* **36**: 2244-2246.

van Ballegooijen M., Koopmanschap M.A., van Oortmarssen G.J., Habbema J.D.F., Lubbe Th.N., & van Agt H.M. (1990). Diagnostic and treatment procedures induced by cervical cancer screening. *Eur J Cancer* **26**: 941-945.

- van Ballegooijen M., van den Akker van Marle M.E., Patnick J., Lynge E., Arbyn M., Anttila A., Ronco G., & Habbema D.F. (2000). Overview of important cervical cancer screening process values in EU-countries, and tentative predictions of the corresponding effectiveness and cost-effectiveness. *Eur J Cancer* **36**: 2177-2188.
- van den Akker van Marle M.E., van Ballegooijen M., van Oortmarssen G.J., Boer R., & Habbema J.D.F. (2002). Cost-effectiveness of cervical cancer screening: comparison of screening policies. *J. Natl. Cancer Inst.* **94**: 193-204.
- van den Akker-van Marle ME, van Ballegooijen M., & Habbema J.D. (2003a). Low risk of cervical cancer during a long period after negative screening in the Netherlands. *Br. J. Cancer* **88**: 1054-1057.
- van Oortmarssen G.J. & Habbema J.D. (1991). Epidemiological evidence for age-dependent regression of pre- invasive cervical cancer. *Br. J. Cancer* **64**: 559-565.
- Walter S.D., Clarke E.A., Hatcher J., & Stitt L.W. (1988). A comparison of physician and patient reports of Pap smear histories. *J Clin Epidemiol* **41**: 401-410.
- WHO (1986). Control of cancer of the cervix uteri. A WHO meeting. *Bull WHO* **64**: 607-618.
- WHO (2002). National Cancer Control Programmes. Policies and Managerial Guidelines, 2nd edition. World Health Organization, Geneva.
- Zappa M. & Ciatto S. (2000). Cervix cancer: Case-control studies on screening. In: *Evaluation and Monitoring of Screening Programmes* (eds Sankila R., Demaret E., Hakama M., Lynge E., Schouten L.J. & Parkin D.M.), pp. 99-118. Europe Against Cancer Programme, Brussels, Luxemburg.

Annex 1

Tables

A. Characteristics of the screening programme

Table A1. Definition of the target population

Catchment area	
Start date of programme (month, year)	
Youngest age targeted for screening	
Oldest age targeted for screening	
Recommended interval between negative tests (in years)	
Groups (if any) not eligible to participate in screening (e.g. hysterectomised)	

Note: Women with a recent Pap smear who fulfil eligibility criteria are included in the group of women eligible to participate in screening.

Table A2. Mode of invitation

Does the programme invite	a ☐ All women in the eligible target population, regardless of Pap-test history? b ☐ All women in the eligible target population, except those who had a recent Pap test (within the past six months or one year)? c ☐ Only the women in the eligible target population who did not receive a Pap test within the recommended screening interval (three or five years)? d ☐ Other, specify _____ e ☐ No invitations are issued
Does the invitation include	a ☐ A pre-fixed, modifiable appointment b ☐ An invitation to get in touch to arrange an appointment c ☐ Other, specify _____
Are non-compliers reminded	a ☐ Yes b ☐ Sometimes c ☐ No

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Table A3. Protocol for repeat cytology

CYTOLOGICAL DIAGNOSIS	PROTOCOL RECOMMENDS REFERRAL OF WOMEN FOR REPEAT CYTOLOGY		
	NO	YES	
		ALL	ONLY SOME WOMEN, SPECIFY
Unsatisfactory			
LSIL			
ASC-H			
AGC			
ASC-US			
OTHER, specify (one line per reason)			

Note: Indicate if repeat smear is recommended. Cross in (X) one box per row.

Table A4. Protocol for referral to colposcopy

CYTOLOGICAL DIAGNOSIS	PROTOCOL INDICATES REFERRAL OF WOMEN FOR COLPOSCOPY				
	NO	YES			
		ALL	ONLY SOME WOMEN		
			AFTER REPEATED TEST	AFTER HPV TRIAGE	OTHER, SPECIFY
INVASIVE CANCER					
HSIL					
LSIL					
ASC-H					
AGC					
ASC-US					
OTHER, specify (one line per reason)					

Note: If Tables A3 and A4 do not suffice to describe your programme's rules for following up screening abnormalities, please provide additional details.

B. Annual tabulations utilising individual screening data

For completion of the following tables, the database supporting the production of results should consist of individual records. It is essential to maintain one record per woman for each screening episode or invitation. Even though the different calendar dates will be kept in the database for different events during the episode, in the following tables the events need to be indexed for each episode using the invitational year, or, if no invitations were done using the screening date.

Data in the tables should be entered for successively smaller subgroups of women. For example, women attending screening include the subset of women referred for colposcopy, and women referred for colposcopy include the subset of women with a histologically confirmed CIN. This approach permits computation of the performance parameters presented in Chapter 7.

For short-term monitoring purposes the following tabulations are based on annually aggregated data. Additional aggregation over different periods of time, particularly over the full recommended screening interval of your programme (3 or 5 years), is recommended. Additionally, use of longer evaluation periods are recommended.

The tables do not show lines or columns for missing values. If missing values exist, please document them and include the women in the row and column totals.

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Table B1. Invitations, coverage by invitation, and status of target population in the cervical cancer screening programme in the year _____

AGE (years)	N RESIDENT WOMEN (a)	INVITED			N ELIGIBLE (c)	% ELIGIBLE 100 x c/a
		N IN THE ABOVE YEAR	N IN THE 3- (5-) YEAR PERIOD (b)	% INVITED (100 x b/a)		
<20						
20-24						
25-29						
30-34						
35-39						
40-44						
45-49						
50-54						
55-59						
60-64						
65+						
OVERALL						

Note:

- a) Resident women are all women residing in the catchment area of the target population in the specified calendar year.
- b) The invitation policy should be specified, e.g., all women in the target population (see Table A1), only eligible women (see Table A1), or only unscreened women (see Table A2), duration of the recommended screening interval (see Table A1).
- c) Eligible women are all resident women (a) except those who are not targeted for screening, e.g. younger or older than age targeted for screening, hysterectomised women, women undergoing assessment or follow-up of treatment. Often, data relevant to eligibility will not be available for all resident women.

The data source should be reported:

- individual linkage with personal identification available, computerized data, specify database
- individual linkage with personal identification available, manual data, specify database
- personal interviews, specify sample, response rate, etc.
- other (specify).

Table B2. Pap smear tests and population coverage with smear tests in the cervical cancer screening programme in the year _____

AGE (years)	N RESIDENT WOMEN (a)	N RESIDENT WOMEN WITH AT LEAST ONE SMEAR					% SMEAR COVERAGE (100 x b/a)
		IN THE ABOVE YEAR	IN LAST 3 (5) YEARS				
			Personally invited	Not personally invited	Invitation status unknown	OVERALL (b)	
<20							
20-24							
25-29							
30-34							
35-39							
40-44							
45-49							
50-54							
55-59							
60-64							
65+							
OVERALL							

Note: Coverage may also be calculated as a percentage of the eligible population.

- The data source should be reported:
- individual linkage with personal identification available, computerized data, specify data-base
- individual linkage with personal identification available, manual data, specify data base
- personal interviews, specify sample, response rate etc.
- other, specify.

Recommended additional tabulation for Table B2: Breakdown of coverage in last 3(5) years by programme status rather than by invitational status, i.e., by performance of smear within organised programme, outside organised programme, or status unknown.

Optional tabulations for Table B2 and subsequent B tables can be added, if relevant:

- 1) Separate tabulations for women attending:
 - initial screening
 - subsequent screening at the regular interval, i.e. in accordance with the routine interval defined by the screening policy (SUBS-R)
 - subsequent screening at irregular intervals (SUBS-IRR).
- 2) Women screened in the given calendar year broken down by invitation status (Invited/ Not invited/Invitation status unknown.)

Table B3. Results of all smears taken in the cervical cancer screening programme in the year _____

AGE (years)	CYTOLOGICAL DIAGNOSIS								OVERALL
	Malignant tumour cells	High grade intraepithelial lesion (HSIL)	Low grade intraepithelial lesion (LSIL)	ASC-H	Atypical glandular cells (AGC)	ASC-US	Negative for intraepithelial lesions	Unsatisfactory	
<20									
20-24									
25-29									
30-34									
35-39									
40-44									
45-49									
50-54									
55-59									
60-64									
65+									
OVERALL									

Note:

If a woman had repeat tests, take all smears into account, but report only one result (the most severe) per woman.

If the above classification is not used, first make your own national table and then convert the results to the above classification.

Recommended additional tabulations for Table B3:

- Enter data for multiple-year periods.
- Enter results of all smears for women with repeat tests, i.e. report more than one smear per woman.

Table B4. Number of women recommended for repeat cytology in the cervical cancer screening programme in the year _____

AGE (years)	REASON FOR RECOMMENDATION						OVERALL
	LOW- GRADE SIL	ASC-H	AGC	ASC-US	UNSATIS- FACTORY	OTHER*	
<20							
20-24							
25-29							
30-34							
35-39							
40-44							
45-49							
50-54							
55-59							
60-64							
65+							
OVERALL							

Note:

The women referred for repeat cytology are included in the screened women (Tables B2 and B3).

Give one reason per woman.

*Other: specify and add an extra column for each different reason.

Optional tabulations can be added for the full recommended screening interval of the programme (3 or 5 years):

- overall compliance to recommendation of repeat cytology
- compliance to recommendation of repeat cytology broken down by reason of recommendation.

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Table B5. Number of women referred for colposcopy in the cervical cancer screening programme in the year _____

AGE (years)	REASON FOR REFERRAL							OVERALL
	INVASIVE CANCER CYTOLOGY	HIGH- GRADE SIL	LOW- GRADE SIL	ASC-H	AGC	ASC-US	OTHER*	
<20								
20-24								
25-29								
30-34								
35-39								
40-44								
45-49								
50-54								
55-59								
60-64								
65+								
OVERALL								

Note:

The women referred for colposcopy are included in the screened women (Tables B3 and B4).

Table B5 includes all referrals, i.e., those arising either from initial or from repeat (follow-up) cytology.

Give one reason per woman.

*Other: specify and provide one column for each different reason.

Table B6. Compliance with referral for colposcopy in the cervical cancer screening programme in the year _____**Period CONSIDERED for colposcopy performance: up to ____/____/____**

REASON FOR REFERRAL (cytology)	N Referred women	Colposcopy performed			Colposcopy not performed
		In referral centres*	In other centres*	Total	
Malignant tumour cells					
HSIL					
LSIL					
ASC-H					
ASC-US					
AGC					
Unknown					
OTHER, specify, one line per reason					

Note:

Include women screened in the stated year who were referred for colposcopy.

Consider all women who underwent a colposcopy after referral and within the date specified at the top of the table to be compliers.

*Distinction by place of colposcopy could be irrelevant given the local organisation.

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Table B7. Cytological and histological results of women who had colposcopy in the cervical cancer screening programme in the year _____

CYTOLOGY	HISTOLOGY								OVERALL
	Invasive Cancer	Adeno Ca in situ (CGIN)	CIN3	CIN2	CIN1	Unsatisfactory	No CIN/CGIN or Cancer	Biopsy not performed	
Malignant tumour cells									
HSIL									
LSIL									
LSIL-CINI									
ASC-H									
ASC-US									
AGC									
OTHER, specify, one line per reason									
Overall									

Note:

Include **only women who were screened in the year stated and who underwent colposcopy**.

Include only one observation per woman, even if more than one colposcopy (or more than one biopsy) was performed.

Indicate the cytology result that was the reason for referral for colposcopy.

Enter the most severe histological finding within a year of the cytology that caused referral.

Koilocytosis is usually classified with CIN1. Any histology different from CIN or Cancer must be included in the 'No CIN/No CGIN/No Cancer' column. Do not include women with unknown result.

Overall values are row or column totals. Except for unknown results, row totals should be the same as for those reported in Table B6 for women having had colposcopy.

Table B8. Women with histologically confirmed CIN or invasive cancer by age group in the cervical cancer screening programme in the year _____

AGE (years)	HISTOLOGICAL DIAGNOSIS									
	Fully Invasive Squamous Ca	Micro Invasive Squamous Ca	Unstaged Invasive Squamous Ca	Invasive Adeno Ca	Other Invasive Ca	Adeno Ca in situ (CGIN)	CIN3	CIN2	CIN1	OVERALL
20-24										
25-29										
30-34										
35-39										
40-44										
45-49										
50-54										
55-59										
60-64										
65+										
OVERALL										

Note:

The women with histologically confirmed CIN or invasive cancer are included in the screened women (Tables B3 and B4).

Enter only one observation per woman even if more than one colposcopy (or more than one biopsy) was performed.

For CIN1, CIN2 and CIN3 column totals should be the same as in Table B7.

The sum of column totals for invasive cancer (columns 2 to 6) should be equal to the column total for “invasive cancer” in table B7.

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Table B9. Treatment performed for CIN/Invasive Cancer in the cervical cancer screening programme in the year _____

TREATMENT	HISTOLOGY						
	Enter the most severe histology before treatment.						
	Invasive Cancer	Adeno Ca in situ (CGIN)	CIN3	CIN2	CIN1	No Biopsy (See and treat)	OVER-ALL
Laser Vaporisation							
Cryotherapy							
Radical Diathermy							
Diathermocoagulation*							
Excision by radio-frequency device (loop, needle, including conisation)							
Cold knife conisation							
Laser conisation							
LLETZ+Laser							
Hysterectomy							
Other: For each treatment not included above, add a line, specifying the treatment.							
Type of treatment unknown†							
Not Treated – no treatment recommended‡							
Not treated – treatment recommended from <3 months‡							
Not Treated – treatment recommended from ≥3 months‡							
Treatment unknown§							
OVERALL							

Note:

Include in Table B9 cases entered in Table B8 (i.e., cases detected in the "screened population"). Some differences with Table B8 may exist because the histology reported there may be from the surgical specimen.

Report the first treatment; and enter also cases treated without previous biopsy and with negative histology of the surgical specimen.

*Diathermocoagulation is not recommended, but it is included in the table in order to recognise if it is performed.

†"Type of treatment unknown": it is known that the woman was treated, but the type of treatment is unknown;

‡"No treatment": it is known that the woman was not treated. Women in this category are divided into 3 groups, depending on the fact that, at the moment data are produced:

- no treatment was recommended
- treatment recommendation was less than 3 months old (possibly not yet performed for practical reasons)
- treatment recommendations was more than 3 months old (woman plausibly non-complying)

§"Treatment unknown": it is not known if the woman was treated.

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Table B10. Cytological follow-up of women treated for CIN2/3 in the cervical cancer screening programme in the year _____

Treatment performed	Interval from treatment ≥6 months			Interval from treatment <6 months
	Cytology = SIL	Cytology = no SIL	Cytology not available	
Laser Vaporisation				
Cryotherapy				
Radical Diathermy				
Diathermocoagulation*				
Excision by radio-frequency device (loop, needle, including conisation)				
Cold knife conisation				
Laser conisation				
LLETZ+Laser				
Hysterectomy				
Other: For each treatment not included above, add a line, specifying the treatment.				
Overall				

Note:

Include women treated for CIN2 or CIN3 or AdenoCa in situ as in Table B9.

Given that tables are expected to be produced yearly, only the first follow-up after treatment (usually after 6 months) is entered. Long-term follow-up of treated women is also recommended (see Chapter 7).

*Diathermocoagulation is not recommended, but it is included in the table in order to recognise if it is performed

3

Methods for screening and diagnosis

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3.1 Executive summary

Screening for cervical cancer requires the use of a test, that is easy to perform by medical or para-medical personnel, is available at an acceptable cost, causes minimal discomfort to the woman and has a high sensitivity and specificity for progressive intra-epithelial lesions (CIN). Evidence of effectiveness should be based on its potential to reduce the morbidity and mortality from cancer. High sensitivity for the detection of CIN is an insufficient criterion for effectiveness, since CIN often regresses. High specificity is required to avoid anxiety, unnecessary treatment and side effects.

The conventional Pap smear partially fulfils these criteria. Cytological screening every three to five years can reduce morbidity and mortality from cervical cancer by 80% or more, if offered in an organised quality-assured setting. The test-validity, in particular the cross-sectional test sensitivity of the conventional Pap smear for CIN, is moderate: between 50 and 70% for CIN; but around 80% for high-grade CIN. Cytological screening in opportunistic settings is less cost-effective and often also less effective.

The occurrence of false-negative and unsatisfactory Pap smears prompted the development of new technologies such as liquid-based cytology and automated screening devices. The quality of the evaluation of the performance of these technologies was often poor and essentially limited to cross-sectional cytological outcomes; verification with a valid gold standard was rarely performed.

Liquid-based cytology (LBC) was originally evaluated using the *split-sample* study design, according to which a conventional smear is prepared from the sampling device prior to the preparation of an LBC smear. Later, the *direct-to-vial* study design was applied which corresponds to the intended use of LBC. Results pooled from *split-sample* studies showed increased detection of LSIL, but not HSIL. However, in *direct-to-vial* studies significantly higher test positivity rates for LSIL and HSIL were observed, whereas the positive predictive value for histologically confirmed CIN2+ (grade 2, or more serious disease) was not lower than for conventional cytology. These findings may suggest increased sensitivity of LBC. However, the level of evidence for this statement is low because of insufficiently controlled and verified study designs. Moreover, pooling the few studies with complete assessment using a gold standard, which permits evaluation without verification bias, did not reveal a statistically significant difference between conventional and liquid-based cytology in test sensitivity or specificity for detecting CIN2+. Randomised controlled trials comparing LBC with conventional cytology which fulfil high standards of diagnostic research and use biopsy-proven outcomes, are still needed. Such studies are currently being conducted. An Italian population-based randomised study recently confirmed that the sensitivity of LBC and conventional cytology are similar.

In general, the proportion of unsatisfactory samples is lower in LBC, and the interpretation of LBC requires less time. The cost of an individual LBC test is considerably higher, but it allows ancillary molecular testing, such as high-risk HPV testing in the case of ASC-US.

One experience with the PAPNET device merits attention, since it was the only randomised clinical trial comparing manual versus automation-assisted interpretation of conventional Pap smears which was designed to use cancer incidence as the final outcome. The preliminary results of this trial showed no differences in detection of cancer or CIN2+. The cross-sectional specificity and positive predictive value were similar as well.

Colposcopy is sometimes proposed as an alternative screening method, but its specificity and probably also its sensitivity are too low for this purpose. Since colposcopy is an essential instrument to orient further diagnostic exploration and treatment, its use is covered more extensively in Chapter 6.

METHODS FOR SCREENING AND DIAGNOSIS

Strong evidence shows that infection with sexually transmittable human papillomaviruses (HPV) is a necessary but insufficient etiological condition for the development of cervical cancer. Furthermore, only high-risk HPV types are associated with cervical cancer. Given this evidence, several applications for HPV DNA detection have been proposed: 1) primary screening for oncogenic HPV types alone or in combination with cytology; 2) triage of women with equivocal cytological results; 3) follow-up of women treated for CIN to predict success or failure of treatment.

A recent meta-analysis concluded that triage of women with equivocal cytological lesions by HPV testing with the Hybrid-Capture 2 assay is more sensitive and equally specific in finding high-grade CIN compared to repeat cytology. Another recent systematic review indicated that HPV DNA detection predicts treatment failure more quickly than cytological follow-up.

The use of HPV detection in the context of primary screening is the focus of intensive research. HPV infections are very common and usually clear spontaneously. Detection of HPV DNA thus carries a serious risk of unnecessary colposcopies, psychological distress and possibly of over-diagnosis. The need to perform cervical cancer screening in an organised programme, rather than in an opportunistic setting, therefore applies particularly to screening based on HPV testing. The specificity of HPV detection could be enhanced by screening only women older than 30 years, due to less frequent HPV clearance in this age group, and by triage of HPV positive women using: cytology; repetition of the HPV test after 6 to 12 months, confirmation of persistence of the same HPV type; identification of high viral loads of the most oncogenic types (in particular HPV 16); and use of standardised HPV detection methods. The high sensitivity of current HPV-DNA detection methods yields very high negative predictive values even for adenocarcinoma precursors that often escape cytological detection. Recent cohort studies indicate a prolonged duration (up to ten years) of the negative predictive value of HPV testing. Nevertheless, further longitudinal research, preferably in an organised setting guaranteeing optimal follow-up, using randomised designs, and targeting relevant outcomes is necessary. Observation of a lower incidence of CIN3+ among screen-negative women in the HPV group compared to the cytology-screened group is an acceptable early surrogate outcome, provided that the potentially increased incidence and need for management of less severe lesions is taken into account.

Population-based randomised trials are now being conducted in several EU member states, comparing HPV screening or combined HPV/cytology screening with cytology screening alone. The observed research outcomes can be analysed further with mathematical modelling in order to define the best policy.

Publication of results of the second round of screening is expected in 2007-2008. Revision of current European screening policy based on cytology should be considered if the randomised trials demonstrate that cumulative incidence of CIN3 and invasive cervical cancer, separately or jointly, is lower in the second screening round in women who were HPV negative compared to women who were cytology negative in the first screening round. The randomized trials will also provide data essential for recommending appropriate screening intervals, target age groups and clinically relevant test thresholds.

Prior to routine implementation of a new screening strategy, the feasibility, cost-effectiveness and quality assurance should be verified and the necessary training and monitoring should be organised. A randomised screening policy, which permits quality-controlled piloting of a new test or procedure in the context of an organised screening programme, is a particularly powerful tool for timely evaluation under real-life conditions.

In a first annex to this chapter, a technical guideline is presented on the preparation of an adequate Pap smear. In a following annex the principles of cytological reporting are discussed using the template of The Bethesda reporting System (TBS). Laboratories should apply only a nationally agreed terminology that is at least translatable into the Bethesda system.

3.2 Assessment of the performance of screening tests: principles and criteria

The rationale of cervical cancer screening is to identify progressive cervical intra-epithelial neoplasia (CIN¹) and, by treatment of recognised lesions, to prevent progression to invasive cancer (Morrison, 1992). The effectiveness of a screening programme depends on the overall programme sensitivity which, in turn, depends on the sensitivity of the respective screening test, the natural history of the disease, and the screening policy (the target age group, screening interval, and procedures for follow-up of positive screens). The essential elements in the natural evolution of the disease are the rates of onset, progression and regression of precursor lesions, and the distribution of their sojourn times. The mean sojourn time of a progressive CIN lesion is at least 10 years and the probability of detection increases as the preclinical phase progresses (Hakama *et al.*, 1985; van Oortmarssen & Habbema, 1991). Therefore, repetition of a moderately sensitive screening test, such as the Pap smear can reduce incidence of and mortality from cervical cancer to a low residual level (van Oortmarssen *et al.*, 1992). The reduction in the cumulative incidence of cancer due to well organised cytological screening every 3 or 5 years is estimated to be 91% and 84%, respectively (Day *et al.*, 1986; Day, 1989; van Oortmarssen & Habbema, 1991).

As explained in Chapter 2, in addition to the properties of the screening test, the success of screening also depends essentially on the participation of the target population and compliance with follow-up, and on the efficacy of treatment of screen-detected lesions. Chapter 3 focuses on various screening test methods. We will describe, and assess the performance of 5 main types of tests that are currently used in cervical cancer screening in Europe or that are proposed as an alternative or supplement to current methods:

1. Conventional Pap smear
2. Liquid-based cytology
3. Automated cytological screening
4. Colposcopy
5. Human papillomavirus nucleic acid detection

Strength of evidence of screening effectiveness

Indicators of screening effectiveness, assessed by different study methods, are enumerated in Table 1 and ranked from high to low according to the level of evidence that such studies provide.

Randomised trials aiming to demonstrate a reduction in invasive cervical cancer provide the highest level of evidence of efficacy of screening. Observation of a lower incidence of cervical cancer in the trial arm in which a new screening test is applied provides the proof that the new method (including the management of screen positives) is more effective than the control method. Nevertheless, conducting such studies requires enormous financial resources and huge study populations which are followed for many years, with a high risk of contamination between the experimental and control arms². Meanwhile, the new technique may become obsolete or no longer be available. Therefore, study of intermediate or surrogate outcomes (e.g., outcomes 4 to 6 in Table 1) has been proposed, as well as simulation of the most likely outcomes relevant to public health using mathematical

¹ In this chapter "CIN" (cervical intra-epithelial neoplasia) is used for histologically confirmed lesions, while the SIL (Bethesda) terminology is used to describe cytological findings.

² Contamination means that study subjects enrolled to participate in a trial arm do not follow the procedures foreseen in the study protocol. For example, women randomised to screening with cytology, may also undergo HPV testing in the context of opportunistic screening.

models. Cohort studies do not yield more rapid results than randomised trials and suffer from several potential biases. Case-control studies which compare screening histories in women with and without cervical cancer are appropriate to evaluate effectiveness retrospectively, but are also prone to several selection and information biases. Changes over time or geographical differences in incidence or mortality can be interpreted as screening effects but can only be accepted as an indication of screening effectiveness if no other factors can plausibly explain the observed changes.

The aim of screening is to prevent cervical cancer, not simply to detect pre-invasive lesions. A new screening test allowing (earlier) detection of more CIN will not necessarily result in more pronounced reduction of cancer incidence because it may only detect additional non-progressive lesions.

Table 1. Ranking of indicators for effectiveness of cervical cancer screening methods by decreasing level of evidence of study outcomes and design

Outcome:

1. Reduction of mortality from cervical cancer, life-years gained.
2. Reduction of morbidity due to cervical cancer: incidence of cancer (Ib+), quality-adjusted life-years gained.
3. Reduction of incidence of cancer (including micro-invasive cancer).
4. Reduction of incidence of CIN3 or worse disease (CIN3+).
5. Increased detection rate of CIN2+ or CIN3+.
6. Increased test positivity with increased, similar, or hardly reduced positive predictive value.

Study design:¹

1. Randomised clinical trial, randomised population based trial.
2. Cohort studies.
3. Case-control studies.
4. Trend studies, ecological studies on routinely collected data.

Cross-sectional test accuracy

Screening requires an accurate test (Wilson & Jungner, 1968): i.e., a test that it is positive when CIN is present and negative when CIN is absent. In other words, a screening test must have a high test sensitivity and specificity. It is important to define the severity of CIN when assessing the accuracy of a test. CIN1 rarely progresses to cancer (Ostor, 1993; Holowaty *et al.*, 1999) and high sensitivity for CIN1 is therefore not particularly relevant. On the other hand, CIN2, and in particular CIN3, indicate a considerable risk of developing cancer and should therefore not be missed by a screening test. CIN2 is an intermediate condition, which contains over-called CIN1 and under-called CIN3 (Sherman *et al.*, 2002). The histological diagnosis of CIN2 is less reproducible than CIN3. Therefore CIN3 should be the preferred outcome in research on the diagnostic accuracy of cervical cancer screening methods. The observation that a new screening test is more sensitive than the conventional test in finding CIN3 provides more evidence that use of the new test in screening will result in a greater reduction in cancer incidence (than if CIN2 were the outcome).

The most comprehensive design for evaluating the cross-sectional accuracy of screening tests is the independent application of all the tests to a screening population, followed by verification in all study subjects, irrespective of the screening test results. Verification should be performed with a valid gold standard, without prior knowledge of the screening test results (Cochrane Methods Group on Systematic Review of Screening and Diagnostic Tests, 1996; Bossuyt *et al.*, 2003). Under these conditions, unbiased estimation of the test sensitivity and specificity is possible.

¹ Only **controlled** studies were considered, i.e., studies which compare two or more screening methods.

Verification bias

Often, even in a research context, only women with positive screening tests and none, or only a few with negative screening test results are verified. This situation results in verification bias, with inflated sensitivity and underestimated specificity. Verification bias decreases, however, if multiple tests are evaluated and at least one of them is very sensitive. Furthermore, referral of a random fraction of screen negatives for the application of the gold standard permits adjustment for verification bias (Begg & Greenes, 1983; Choi, 1992; Irwig *et al.*, 1994; Ratnam *et al.*, 2000; Pepe, 2003).

Application of two screening tests to the same study subjects, and verification with an acceptable gold standard in all subjects who are positive for one or both tests, permits unbiased estimation of the tests' positive predictive values, relative sensitivities and the detection rates of true positives (Schatzkin *et al.*, 1987; Chock *et al.*, 1997)¹⁻². The same unbiased test accuracy parameters can be derived from the baseline results of randomised clinical trials in which different tests are applied to different subjects, after completion of the first screening episode.

Quality of the gold standard

Assessment of the gold standard, based on the screening test result, includes a serious risk of overestimation of both the sensitivity and specificity. Verification should therefore be performed independently in diagnostic research evaluating the cross-sectional accuracy of a screening test. This can be difficult when the screening test and the gold standard are based on the same principle, for instance, when VIA screening (visual inspection of the cervix after application of acetic acid), is validated using colposcopy.

It is usually assumed that histological examination of material obtained by colposcopically directed biopsy, loop excision or endocervical curettage, and – in absence of biopsy – a negative colposcopic assessment provide valid ascertainment of the true disease status. Recent data indicate that this assumption may not be true (Pretorius *et al.*, 2006; Gage *et al.*, 2006).

When the prevalence of disease is low, an *approximated test specificity* can be computed, even without systematic verification of a random sample of test-negatives, from the ratio of the number of test-negatives over the total number of study subjects minus the true positives (Morrison, 1992). ($\text{Specificity}_{\text{approx}} = \# \text{ test negatives} / (N - \# \text{ true positives})$; where N = the number of all tested individuals).

The reliability or reproducibility of a test expresses the capacity to obtain the same test result – correct or not – when the screening test is repeated on the same individual. The reliability depends on the definition of distinct test criteria that can be applied by skilled personnel. Poor reproducibility automatically yields low average sensitivity and specificity. Reproducibility can be enhanced by training and quality control. Evaluation of new screening tests requires reproducibility experiments, preferentially including investigations under field conditions.

Longitudinal sensitivity

Once again, the observation of increased cross-sectional sensitivity of a new test for histologically confirmed CIN does not necessarily imply that, in a screening programme, the new test would yield a reduction in incidence of lethal cervical cancer compared to conventional cytological screening³. Nevertheless, if biological and epidemiological arguments justify the assumption that the lesions de-

¹ The same is true when different tests are studied in different populations as long as the prevalence of disease can be assumed to be the same (e.g. in randomised trials) (Morrison, 1992).

² When not all screen-positives are verified and the selection of verified positive cases is not random, verification bias still can occur at the level of the PPV, detection rate and relative sensitivity.

³ It is important to distinguish cross-sectional and longitudinal accuracy parameters. Increased detection with a new test of small CIN2 that will largely regress, will result in higher cross-sectional sensitivity, which is not clinically useful (over-diagnosis). On the other hand, a cross-sectionally false case may be longitudinally true positive; for example, a screen-positive woman who currently does not have colposcopically visible CIN, may develop a high-grade lesion in the future.

tected in excess by the new method have substantial likelihood of progression (acceptable longitudinal positive predictive value) and if screen negatives have a substantially lower chance to develop cancer in the future (higher longitudinal negative predictive value), evaluation of the new test in a randomised population-based trial, preferentially in an organised setting can be considered (see Chapter 2). Simulation models should be performed to define the best policy for application of the new test.

Costs of screening

The above discussion deals essentially with the influence of test sensitivity on programme effectiveness. However, cervical cancer screening is implemented in large populations and is therefore costly. Costs are largely determined by the test specificity. Table 2 presents an overview of the cost components attributed to screening.

A small decrease in specificity can have dramatic consequences on costs. The number of additional false positives is computed from nearly the complete target population, since the prevalence of progressive cervical cancer precursors is low. Nevertheless, the loss in specificity of a screening test can be limited by extending the duration of the screening interval, by increasing the age at onset of screening and by elevating the cut-off for test positivity. Reliable mathematical models can be used to estimate the final outcome per unit of cost.

Table 2. Overview of cost components of a screening programme

1. Cost price of the screening test (investment and recurrent costs); fees of health professionals (time for preparation, interpretation of the screening test, documentation, training); logistical costs (transport, processing, storage); administrative costs (invitation, registration and analysis of data).
2. Specificity of the screening test: cost of follow-up and treatment of women with false-positive results or having non-progressive screen-detected lesions (over-diagnosis).
3. Sensitivity of the screening test (longitudinal): cost for follow-up and treatment of true positives; this cost may be off-set by cost savings in avoided treatment of advanced disease.
4. Human costs: time spent by women to be screened, anxiety and discomfort of follow-up and/or treatment of women with true and false-positive results and consequences of delay in detection of cancer in false-negative women.
5. Specificity of quality control, triage and diagnostic follow-up procedures, contributing to increased positive predictive value and savings by avoiding treatment of false-positive women.
6. Quality of screening test procedures; satisfactory rate influencing the need for repeat tests.

Section 3 starts with a description and evaluation of the current standard screening test, which is the conventional Pap test. In the next sections, the newer alternatives of cytological screening using liquid-based cytology and automated screening devices are addressed. Subsequently, colposcopy is described only briefly since it is not an appropriate screening instrument. Colposcopy is dealt with more completely in Chapter 6 in the framework of management of women with cytological abnormalities.

Finally, some methods of HPV testing, which are applicable in high-through-put, routine settings, are enumerated and their performance is evaluated in three possible settings: 1) primary screening; 2) triage of minor cervical lesions and 3) follow-up after treatment of high-grade CIN.

3.3 Conventional cervical cytology

3.3.1 Description of conventional cervical cytology

3.3.1.1 Principles of conventional cytology

A sampling device is needed to collect cells from the surface of the uterine cervix and the cervical canal. Cells are either directly smeared on a glass slide or deposited on a slide after being first transferred to a liquid medium (see technical guideline in appendix 1: preparation of an adequate Pap smear). For microscopic evaluation by a cytologist the cells must be stained. The cells are then analysed using a microscope.

3.3.1.2 Reading a cervical smear

Reading a cervical smear is a rather complex procedure. Hundreds of thousands of cells cannot all be evaluated visually in detail. The problem of searching for a few atypical cells in a large area led to the development of the cyto-technologists' profession. This chapter will address both the localisation phase and the interpretation phase of cervical cytology. In reality, the two phases cannot be easily separated.

3.3.1.3 Screening technique and localisation

Magnification

The resolution of the unaided human eye is about 100µm. Considering a nuclear size of 10µ, a ten power magnification is about the minimal enlargement required if regular sized nuclei are to be detected at all. At this magnification, no nuclear detail can be recognised. For screening purposes a 10-power objective and a 10-power eyepiece magnification are used. At this magnification, nuclear features are mainly size and contrast, whereas structural resolution is very poor even after foveal fixation. Lower magnification can be used for orientation, but not for screening in gynaecological cytology. Higher magnifications of 25X and 40X are used to view objects of interest in more detail.

Slide movement

Generally, the screening of a case starts on one edge of the cover glass. After the inspection of the field of view, the observer passes on to the next field of view with a quick movement of the stage. This process of alternating movements and stops is continued in the same direction until the opposite side of the cover slip is reached. Here the observer moves to the next line where the screening is continued in the opposite direction. In this way the slide is screened in a "meander"-like fashion until the total area of the slide has been screened.

Physiology of visual microscopic perception

During about 180 msec the slide is moved from one field of view to the next. During this time there is no foveal fixation. The new field of view is examined during the latency period by peripheral vision. If no conspicuous object is found, after about 230 msec the microscope stage is moved to the next field of view. If there is a conspicuous object, it will be fixed by the fovea after a very rapid eye movement, a saccade. If necessary, in the same field of view several objects will be fixed, each after a saccadic eye movement. Then the stage will be moved to the next field of view. This process shows some obvious limitations in screening performance. Only a limited part of the specimen area is analysed with stationary fields of view. During stage movement no fixation takes place. Most of the area can be covered only by peripheral vision.

Screening duration

The relation of total screening time, number of fields of view and the slide area in stationary fields of view can be calculated. There is a correlation between total screening time and the specimen area that can be viewed during the stops in the screening process. With less screening time per specimen, only part of the total area is seen. The use of special cell preparation techniques like LBC resulting in deposition of the representative sample with a randomised distribution in a limited slide area is at present the only acceptable approach to substantially reduce the deposition area. If the time spent by a cyto-technologist on a case is evaluated, not only the screening time but also some time for documentation of the screening results must be taken into account. A cyto-technologist needing on average five minutes per slide and one minute for documentation, will be able to read 10 cases per hour and 60 cases per day in six hours spent at the microscope. Of this time, about 60 minutes will be used for reading patient documents and filling in forms.

3.3.1.4 Cytological interpretation and reporting

The basic assumption of cytological diagnosis is that it is related to the histology of the relevant tissue. This means that there is an equivalent appearance of cells even after the cells are detached from tissue and all three-dimensional information is lost.

Cytological findings should be categorised according to an established reporting system which should at least be translatable into TBS (see Annex 2).

3.3.1.5 Clinical applications of cervical cytology

Conventional cytology is still the standard method for primary cervical cancer screening. Repetition of the Pap smear is used as a triage method in case of minor cytological abnormalities and as a follow-up method after treatment of lesions (Chapter 6).

3.3.1.6 Quality of conventional smears

The judgement of the quality of a smear is an essential component of the cytological interpretation of a Pap smear. The criteria for considering a Pap smear as satisfactory or unsatisfactory are discussed in Section 3.5, after the evaluation of LBC.

3.3.2 Performance of conventional cervical cytology**Programme sensitivity**

The efficacy of conventional cytological screening for cervical cancer has never been demonstrated in randomised clinical trials (design type 1, see Table 1) but evidence of its effectiveness is nowadays widely accepted from observational studies (design types 2-4). An overview of the evidence is provided in the systematic review performed by the International Agency for Research on Cancer in 1986 and updated in 2005 (Hakama *et al.*, 1986; IARC, 2005). These reviews concluded that three-to-five-year screening in women 35-55 years old after 2 previous negative smears in an organised setting yields a reduction in cumulative incidence of squamous cervical cancer of 91% to 84% (Day *et al.*, 1986). The programme sensitivity is lower and more heterogeneous in non-organised than organised settings due to lower and more variable test sensitivity (less rigorous quality control). The duration of low risk associated with a negative smear result is lower in women younger than 35 years (Sasieni *et al.*, 1996). More estimates of the relative protection (a performance parameter di-

rectly related to programme sensitivity) offered by cytological screening, are shown in tables 2 to 5 in Chapter 2.

Cross-sectional test accuracy of cervical cytology

The cross-sectional test validity of cervical cytology for CIN using the histological result of a biopsy, conus, endo-cervical curettage or hysterectomy as gold standard, was evaluated in two meta-analyses (Fahey *et al.*, 1995; McCrory *et al.*, 1999; Nanda *et al.*, 2000). Data extracted from the most recent American meta-analysis have been pooled and reanalysed, yielding estimates of accuracy that are summarised in Table 3.

Table 3. Meta-analysis of test sensitivity and specificity of cervical cytology at three test thresholds (ASCUS+, LSIL+ and HSIL+) for colposcopically or histologically confirmed presence of CIN2+ or CIN1+ pooled from studies with complete and incomplete gold standard verification¹

a. Outcome: presence of CIN2+

All studies

Test threshold	Sensitivity	(95% CI)	# Studies	Specificity	(95% CI)	# Studies
LSIL+	0.83	(0.80-0.90)	46	0.61	(0.55-0.67)	46
HSIL+	0.58	(0.49-0.66)	45	0.89	(0.87-0.90)	45

Only studies without verification bias

Test threshold	Sensitivity	(95% CI)	# Studies	Specificity	(95% CI)	# Studies
LSIL+	0.77	(0.58-0.97)	6	0.92	(0.89-0.95)	6
HSIL+	0.87	(0.78-0.96)	1	1.00	(0.99-1.00)	1

b. Outcome: presence of CIN1+

All studies

Test threshold	Sensitivity	(95% CI)	# Studies	Specificity	(95% CI)	# Studies
LSIL+	0.67	(0.63-0.71)	72	0.73	(0.71-0.76)	72
HSIL+	-	-	0	-	-	0

Only studies without verification bias

Test threshold	Sensitivity	(95% CI)	# Studies	Specificity	(95% CI)	# Studies
LSIL+	0.52	(0.38-0.66)	9	0.96	(0.94-0.98)	9
HSIL+	-	-	0	-	-	0

There is a tendency toward higher sensitivity and lower specificity for CIN2+ than for CIN1+. The sensitivity decreases and the specificity increases with higher cytological test threshold. As expected, studies without verification bias, in which all subjects were submitted to the gold standard, showed lower sensitivity and higher specificity.

The low pooled sensitivity of cytology at the threshold of LSIL+ for the presence of CIN1+ found in studies with complete verification (52%; 95% CI: 38-66%) has been cited as justification for yearly as opposed to less frequent screening, or for introducing newer, more sensitive methods. This level of sensitivity is also frequently cited in the literature. It should be considered, however, that CIN1+

¹ Adapted from McCrory D.C., Matchar D.B., Bastian L., Datta S., Hasselblad V., Hickey J., Myers E., & Nanda K. (1999). Evaluation of cervical cytology. AHCPR Publication No. 99-E010, 1-274. Rockville (MD), USA, AHCPR

is the outcome on which this level of sensitivity is based. CIN1 lesions usually regress or, if progressive, have a high chance to be detected while still non-invasive, at a subsequent screening. Studies included in the meta-analysis, particularly those with complete gold standard verification, often involved follow-up settings not representative of a screening situation. The test sensitivity of cytology for CIN estimated by modelling from the historical British Columbia cohort (without definition of test and outcome thresholds) was 80% (Boyes *et al.*, 1982; van Oortmarssen & Habbema, 1991). This is an estimate of sensitivity evaluated in an organised screening setting with good quality control.

Cuzick *et al.* 2006 recently pooled data from selected studies, conducted in Europe and North America, where both the accuracy of cytology and HPV screening for detecting underlying CIN2+ were assessed. The pooled sensitivity of cytology (cut-off not documented) was only 53% and the specificity 96%. The low sensitivity was mainly due, however, to the outlying values observed in 3 German settings¹.

In conclusion, the test sensitivity and specificity of the conventional Pap smear are not known precisely. The sensitivity for CIN2+ at low cytological thresholds is, on average, relatively high (in the range 70-80%), but it also can be considerably lower in certain situations. The estimation of the accuracy varies by the characteristics of the study group (age, screening history, screening or follow-up context) and the study design (selection bias, definition of cut-offs, method of gold standard assessment, verification bias, independent assessment of gold standard).

Despite potential variation in test performance, there is convincing evidence of the effectiveness of cytological screening, if offered in a well organised setting with quality control at all levels.

3.4 Liquid-based cytology

3.4.1 Description

Thin-layer cytology or liquid-based cytology (LBC) is a new technique for transferring the cellular material to the microscope slide. The cervical broom is usually recommended for taking the sample. However, a plastic extended-tip spatula or the combined use of plastic spatula and endocervical brush are also options.

The smear is not transferred in the usual way onto a slide (see Annex 1). The sampling device carrying the material is immersed in a container with a special liquid transport medium. The container is then sent to a specially equipped laboratory.

Several commercial systems have been developed in the last fifteen years, among which ThinPrep (Cytyc, Boxborough, MA, USA) and the BD SurePath™ System (formerly, AutoCyte PREP, BD Diagnostics, Diagnostic Systems - TriPath USA) are the most well-known. With the ThinPrep-2000 or the more fully automated ThinPrep-3000 processor, the liquid is aspirated through a membrane that detains the cellular material, which is then stamped onto a slide in the form of a very thin layer, often called a *monolayer*. The sample collected with the BD SurePath™ system undergoes a proprietary Cell Enrichment™ process, which removes obscuring cellular material and debris (blood, mucus and inflammatory cells) which could obscure the view of diagnostically relevant cells. Only

¹ Another meta-analysis involving more European/North American studies yielded a pooled sensitivity of cytology at cutoff ASCUS+ for finding CIN2+ of 70% (95% CI: 60-83%). After omission of the German studies, the pooled sensitivity increased to 78% (95% CI: 69-87%) (Koliopoulos *et al.*, 2006 & 2007; Arbyn *et al.*, 2006).

ThinPrep and BD SurePath™ have so far been approved in the United States by the Food and Drug Administration (FDA). Other fluid-based systems are manufactured as well, such as: CYTOscreen System® (Seroa), Turbitec® (Labonord), PapSpin® (Shandon), Cytoslide® (Menarini), and SpinThin® (Shandon).

Until recently, the FDA only allowed ThinPrep to claim lower inadequacy rates and higher detection rates of LSIL and HSIL in comparison with conventional cytology. For AutoCyte, only the label of improved preparation quality and equal detection of cytological abnormalities was permitted. In May 2003, the FDA approved the claim of increased HSIL detection with BD SurePath™ as well.

3.4.2 Rationale for liquid-based cytology

A first advantage attributed to fluid-based methods is that almost all the sampled cells are rinsed into the liquid while with the conventional smear a selective portion of the cellular material may remain trapped on the sampling device (Rubio, 1977). Transfer via a fluid medium increases the likelihood of representative smears (Hutchinson *et al.*, 1994). The ThinPrep and SurePath systems produce circular areas that contain an average of 50,000 to 75,000 randomly selected cells, whereas the conventional Pap smear usually contains 100,000 to 250,000 cells, which is about one-fifth of the cellular material available on the sampling device (Hutchinson *et al.*, 1994).

Fixation of the cell material is optimal in LBC. However, the altered background requires training and a period of adaptation for the cytologist (Austin & Ramzy, 1998). Red blood cells and mucus are for the most part absent and leukocytes are more evenly distributed. Epithelial fragments, which are difficult to interpret on a classical smear, are for the most part disaggregated during the preparation, while diagnostic clusters of columnar or metaplastic cells are usually preserved. The microscopic visualisation of a calibrated thin layer of properly distributed cells is more comfortable for cytological interpretation, which should facilitate the evaluation of cytological structures (Linder & Zahniser, 1997; Austin & Ramzy, 1998).

Multiple smears can be made or additional investigations performed on the residual fluid (e.g. DNA detection of the human papillomavirus or Chlamydia) without the necessity to recall the woman concerned (Sherman *et al.*, 1997; Ferenczy & Franco, 1997).

A thin-layer specimen might be a more proper target for automated screening devices.

A considerable barrier is the higher cost - both the capital investment and the operating costs - and the dependence on the manufacturer's disposables.

3.4.3 Recent reviews, meta-analyses and pilot studies

Several reviews and meta-analyses of the performance of LBC have been carried out over the last 5 years. Conclusions formulated by the reviewing authors were disparate and depended largely on selection criteria to include individual studies and the considered performance parameters. Studies comparing test positivity rates for low-grade cytological abnormalities often yielded more favourable results for LBC, whereas in studies focusing on accuracy for biopsy-confirmed high-grade CIN, no significant differences between conventional and LBC were found.

For this reason it was decided to conduct an exhaustive meta-analysis in the framework of the evaluation of new screening methods, which was one of the priorities of the European Network for Cervical Cancer Screening (Arbyn & Abarca, 2003). The results of this meta-analysis, including reports published between 1991 and 2005, are summarised below.

3.4.3.1 Comparison of the test characteristics of liquid-based cytology with the conventional Pap smear

Multiple meta-analyses including studies comparing the conventional Pap smear (CP) with LBC were performed using low-level and progressively higher-level inclusion criteria. Two separate study designs were distinguished: 1) concomitant testing in which a CP and an LBC are prepared at the same time from each woman¹ and 2) 2-cohort design, according to which CP and LBC samples are taken from separate but comparable populations.² The lowest-level outcomes were differences in cytological test positivity rates and quality judgment. The highest outcome was diagnostic validity for histologically confirmed CIN2+ for which all cases, including cytologically negative cases, were submitted to colposcopy, and biopsy if suspicious colposcopy. In total 108 reports from 93 studies were included in the meta-analysis of test positivity or quality but only six reports permitted unbiased assessment of sensitivity and specificity.

Differences in test-positivity rate between LBC and CP

- **Studies with concomitant testing (n=48)**

More LSIL lesions were detected with LBC than CP: on average, 12% (95% CI: 7-17%) more in ThinPrep slides and 27% (95% CI: 9-48%) more in AutoCyte/ SurePath slides.

The pooled test positivity rate ratios for HSIL+ and ASCUS were not statistically different from unity.

- **Two-cohort studies (n=35)**

More LSIL and HSIL lesions were detected with LBC, and the observed pooled differences were always statistically significant and substantially higher than in studies with concomitant testing.

For ThinPrep: 61% (95% CI: 34-93%) more HSIL and 77% (95% CI: 54-103%) more LSIL. For AutoCyte/SurePath: 47% (95% CI: 18-94%) more HSIL and 52% more (95% CI: 31-76%) LSIL. Positivity rates for ASCUS were similar.

Test positivity rate ratios are considerably higher in direct-to-vial studies, suggesting bias in split-sample studies with disadvantage for LBC. Spreading of cellular material for a conventional Pap smear preparation might cause selective removal of diagnostic elements that are

subsequently no longer available for LBC. Possible non-comparability of populations in direct-to-vial studies (only one was randomised) or classification bias due to systematic over-interpretation by cytologists having converted to LBC are other plausible explanations.

Positive predictive value

Increased detection of cytological abnormalities (ratios >1) provides insufficient evidence for improved sensitivity of LBC compared to CP. Therefore verification with a valid gold standard is needed. Differences in positive predictive value, considered at a given cytological cut-off, indicate

¹ Most often, in studies with concomitant LBC and CP testing, a conventional CP is prepared first and subsequently the residual cellular material on the sampling device is used to prepare an LBC (split-sample studies).

² In 2-cohort studies, all cellular material on the sampling device is transferred to the vial (direct-vial studies) in the LBC cohort. In general, the comparison is simply historical: before vs after introduction of LBC.

whether higher cytological test positivity rates in LBC are due to an increase in false-positive results.

Biopsy rates (the proportion of abnormal slides with biopsy confirmation) were always balanced in split sample studies: this means the biopsy rates were nearly equal within a given study for cases with a positive LBC and/or CP smear. The positive predictive value ratio (PPV_{LBC}/PPV_{CP}) never differed from unity in split sample studies regardless of histological outcome or cytological cut-off, with the exception of AutoCyte at cut-off LSIL+ for an outcome of CIN2+. In this exception, the pooled PPV of LBC was lower than that of CP (ratio: 0.92; 95% CI: 0.85-1.00). Nevertheless this difference was only marginally significant ($p=0.047$). The result of similar PPV was quite robust: most often the inter-study heterogeneity was not significant and the results did not vary with the level of completeness of biopsy information.

Two-cohort studies were often unbalanced with respect to the biopsy rates. The inclusion of studies with large differences in completeness of biopsy verification in LBC and CP cohorts might result in serious biases if the PPVs were different in both types of smear preparation.

For the meta-analysis reported here, the relative PPV was pooled only from 2-cohort studies in which the biopsy rate was higher than 80% in both comparison groups, or in which the difference in biopsy rates between LBC and CP was less than 10%.

The relative PPVs were never significantly lower than one. Moreover, for ThinPrep smears, the PPV defined at HSIL+ for an outcome of CIN2+ was a significant 7% higher than for CP (ratio: 1.07; 95% CI: 1.02-1.12). For AutoCyte, the relative PPV was significantly higher when the threshold was LSIL+ and the outcome was CIN2+, and when the threshold was ASCUS+ and the outcome CIN1+.

Numerous studies were excluded by definition, because they did not reach 80% completeness in histological verification of positive smears, or because the difference in completeness was >10%. In 2-cohort studies, verification is - by definition - not blinded towards the preparation method, increasing the risk of reporting bias.

Accuracy (sensitivity and specificity) for CIN1+ and CIN2+

Verification of all study cases was only performed in 5 studies with concomitant testing (Ferency *et al.*, 1996a; Ferency *et al.*, 1996b; Bergeron *et al.*, 2001; Coste *et al.*, 2003; Confortini *et al.*, 2004) and in one 2-cohort study (Taylor *et al.*, 2006). Given the lack of differences between commercial preparation systems, we pooled data from all LBC systems.

Improved accuracy of the LBC systems for detection of histologically confirmed CIN2+ could not be concluded from the meta-analysis: pooled sensitivity and specificity ratios were not significantly different from unity. Coste *et al.* (2003) even concluded that the conventional Pap smear was superior to LBC, but this conclusion was based on the outcome of CIN1+.

Accuracy ratios were recomputed, including studies with at least 80% confirmation of screening test positives and 5% of cases with a normal screening test result. Assuming verification of cases was random, we computed extrapolated accuracy measures adjusted for verification bias (Begg & Greenes, 1983). Again, no statistically significant difference between LBC and CP was evident in sensitivity or specificity for CIN2+ (95% CI always included unity). A tendency toward increased sensitivity for CIN2+ was essentially due to one outlying result (Confortini *et al.*, 2004). Because of inter-study heterogeneity, the difference was not significant. At the other cut-offs (HSIL+ and LSIL+) results were not significantly heterogeneous (p for Cochran's $Q > 0.15$)¹.

¹ A recent report of the baseline results of a population-based randomised trial conducted in Italy, comparing screening with conventional cytology with LBC and HPV testing, showed increased cytological test positivity

3.4.3.2 Comparison of the adequacy of liquid-based and conventional smears

No significant reduction in the pooled inadequacy rate was observed in the split-sample studies, whereas in direct-to-vial studies this reduction was more pronounced and almost significant for ThinPrep (rate ratio: 0.66; 95% CI: 0.42-1.02; 23 studies) and significant for AutoCyte/SurePath (ratio: 0.17; 95% CI: 0.10-0.32; 11 studies). There was significant inter-study heterogeneity, which can be attributed to poor standardisation in definition and application of quality definitions. The same contrasts were observed for the proportion of suboptimal or SBLB (satisfactory but limited by) smears.

Most studies did not report complete quality information. The proportion of smears obscured by blood or inflammatory material or inadequate fixation was reduced in LBC and this was significant in most situations. The proportion of inadequate smears due to scanty cells was significantly higher in LBC series using the split-sample design. On the other hand, in direct-to-vial studies, the proportion of slides with poor cellularity was significantly lower in SurePath/AutoCyte slides (ratio: 0.13; 0.02-0.32) than in conventional Pap smears.

In split-sample studies, LBC slides showed a significantly higher proportion of smears with endocervical cells (EC-) compared with conventional smears. On the contrary, in direct-to-vial studies, the percentage of EC- in LBC was similar (in case of ThinPrep) or lower (in case of SurePath/AutoCyte) than in conventional cytology, confirming the disadvantage of LBC using the split-sample study design.

Keeping the sampling brush in the preservation liquid may be beneficial for cellularity and the presence of endocervical cells (Bigras *et al.*, 2003).

3.4.3.3 Pilot projects conducted in Scotland and England

In a pilot study, conducted by the *Scottish Cervical Screening Programme* (2002), smear takers were randomised into two groups, collecting conventional Pap smears and ThinPrep preparations, respectively. Smears and vials were sent to 4 laboratories at which cyto-technologists had been trained to interpret LBC smears. Significantly more moderate and severe dyskaryosis lesions were found in ThinPrep and significantly fewer LBC preparations were judged inadequate. The positive predictive values (at cut-off HSIL+, E. McGoogan, personal communication) were similar for both preparation techniques.

The NHS demonstration project (England)

As part of a pilot project conducted in 3 selected laboratories, LBC was introduced, after a learning transition period of 3 to 6 months (Moss *et al.*, 2003). In two laboratories, the ThinPrep system was introduced and in another the SurePath. The proportion of inadequate smears dropped significantly from 9.7% (95% CI: 9.4-10.0%) to 2.0% (95% CI: 1.8-2.2%) in the two laboratories in which ThinPrep was used and to 0.9% (95% CI: 0.8-1.1%) in the laboratory which applied SurePath. In the ThinPrep laboratories significantly more SIL and HSIL lesions, and in one of them (lab C) more borderline lesions were also found. In the laboratory using SurePath, fewer HSIL and borderline lesions were detected. The excess of lesions detected with LBC was concentrated in the age group 20-34 years.

Sensitivity and specificity for histologically confirmed high-grade CIN were documented in neither of two pilot projects. Nevertheless, based on higher detection of cytological high-grade lesions and

rates, no statistically significant increase in sensitivity and a statistically significant lower PPV for CIN2+ (Ronco *et al.*, BMJ 2007: 335:28).

lower inadequacy rates, and in light of cost-effect simulations, the respective health authorities in Scotland and England decided to convert completely to liquid-based cytology (Mayor, 2003).

3.4.3.4 Influencing factors

Multi-variate meta-regression analysis revealed that the gain in detection of HSIL+ and LSIL+ in 2-cohort studies was significantly higher in studies published before 2001 and in studies where cytologists were trained in interpretation of LBC smears just before the onset of the LBC cohort. Inclusion of other assessed covariates did not contribute significantly to explaining inter-study heterogeneity. The assessed factors were: composition of the study population, clinical setting (screening, follow-up or mixed), the version of the LBC-system (betaTP, TP2000, TP3000; CytoRich, AutoCyte PREP, SurePath, other LBC systems), collection devices, training of smear takers, blinding of screeners, reviewers, colposcopists, and histologists, quality control of cyto-technologists' first diagnosis, definition and completeness of gold standard verification, length of follow-up period, and, last but not least, the disclosed interests of the researcher and involvement of the manufacturers of devices. Nevertheless, an influence of some of these factors on study outcomes cannot be excluded, given the generally poor reporting of study details.

3.4.3.5 Economical aspects of liquid-based cytology

Time was measured in 10 studies included in the aforementioned meta-analysis of screening. The simple mean was 237 and 338 seconds, respectively, for thin-layer and conventional Pap smears (reduction of nearly one-third) (Arbyn *et al.*, 2005). The need to adjust the microscope objective is eliminated in LBC since the cellular material is localised in only one very thin layer (Payne *et al.*, 2000). McGoogan reported that interpretation of thin-layer specimen was more tiring (McGoogan & Reith, 1996). Papillo remarked that the shorter evaluation time was off-set partly by a longer processing/preparation time (Papillo *et al.*, 1992). This remark is no longer valid for the more automated processors. In the UK demonstration project, introduction of LBC was considered cost-effective essentially due to economical reasons: reduction in the proportion of inadequate smears and subsequent decline in repeat smears¹ and the shorter interpretation time (Moss *et al.*, 2003).

3.4.3.6 Training and time-trend effects

The introduction of LBC requires adequate training of cyto-technicians. Results reported in the literature often reflect performance during the first months after introduction of LBC, when cytologists are still not sufficiently accustomed to the new technique (McGoogan, personal communication). A learning effect was demonstrated in the NHS demonstration trial, with higher abnormality rates during the first two months of the pilots (Moss *et al.*, 2003).

3.4.4 Recommendations for future research

Future research should apply more rigidly controlled study designs: i.e., randomised trials (conventional versus liquid-based methods), in representative screening settings, with clear definitions of study outcomes, including validation of test results by acceptable gold standards, blinded to the trial arm, and providing detailed information on other factors potentially influencing study results. Differences in detection rates of histologically confirmed CIN2+ or CIN3+ would be an acceptable

¹ The economic savings brought about by the reduction in unsatisfactory smears is expected to be substantially lower in other EU member states, since the inadequacy rate is substantially lower in the rest of Europe.

intermediate study outcome. Studies assessing the adequacy of slides should cover all components of the quality judgement: cellularity, composition, preservation, aspect, and presence of obscuring elements. Currently, such trials are being conducted in the Netherlands and Italy.

Studies of costs and effects and cost-effectiveness ratios (cost per additional case of CIN2+ detected) should be extended, using mathematical modelling, towards more relevant outcomes such as costs per avoided case of cancer or life-year saved. Cost-effectiveness studies should be based on the best available and precise local financial information, accurate estimates of effects derived from trials and updated meta-analyses, and plausible assumptions on the natural history of cervical cancer.

3.4.5 Conclusions

The meta-analysis reported in this section provides some support for the hypothesis that the split-sample study design creates a bias that puts LBC at disadvantage. Detection of HSIL was similar in LBC and CP. However, two-cohort studies showed substantial increases in detection rates of LSIL and HSIL in liquid-based smears, whereas the positive predictive value for moderate dysplasia or more severe disease was not reduced significantly in comparison with conventional Pap smears. From this observation, a gain in sensitivity without loss in specificity could be assumed. However, the level of evidence for this deduction is low, because of insufficiently controlled study designs and the high probability of selection and expectation biases. Moreover, studies with complete verification, including one 2-cohort study, indicated similar sensitivity and specificity for high-grade CIN. Consequently, no evidence is currently available indicating superior test performance of fluid-based cervical cytology. Nevertheless, there is evidence that the test performance of LBC is equivalent to CP.

The quality of LBC samples is superior to that of CP. In the UK, the proportion of inadequate samples decreased substantially with LBC, making LBC an efficient option. Elsewhere the impact on the number of unsatisfactory smears has generally been low. The interpretation of LBC smears requires less time.

No evidence is available indicating higher accuracy of LBC for high-grade cervical intra-epithelial neoplasia. Nevertheless, six studies with complete colposcopy and or biopsy verification provide evidence indicating equal cross-sectional sensitivity and specificity for both preparation systems. Therefore, implementation of LBC in screening should be based on cost considerations and local feasibility. Further research comparing the performance and cost-effectiveness of CP and LBC should be conducted in well-planned trials.

3.5 Quality of the cervical smear

TBS criteria for adequacy

Reporting of sampling quality was one of the most innovative proposals made in 1988 by the Bethesda terminology (National Cancer Institute, 1989). Three categories were proposed: satisfactory, satisfactory but limited, and unsatisfactory.

The second category was mostly used for smears not containing endocervical or metaplastic cells (an indicator for sampling the transformation zone), or for partial inflammatory smears. This category was later eliminated since clinicians felt obliged to repeat the smears. Currently, in addition to reporting the interpretation of smears, the presence of fewer than 10 endocervical cells or inflammatory exudates obscuring cells (in less than 75% of the smear) should be documented. The exclusive presence of columnar cells (with no squamous cells) indicates a non-representative smear. Furthermore, the clinician must make the decision as to whether or not a new smear should be taken.

If a smear is considered unsatisfactory, the reason must be noted, and information about transformation zone sampling should also be provided.

Cellularity

According to TBS, at least 8,000 to 12,000 squamous cells must be present in a conventional smear (Solomon *et al.*, 2002). This minimum cell range should be estimated and laboratories should not count individual cells. TBS provides photomicrographs as “reference images” of known cellularity (Solomon & Nayer, 2004). There are few evidence-based criteria for adequacy of conventional smears, but the organised programme in the UK was based on the concept that cervical smears should consist of clearly displayed cellular material covering one-third of the microscopy slide and preferably over one-half (BSCC Editorial, 1990) but later guidelines have been less specific (NHSCSP, 2000).

TBS proposes at least 5,000 squamous cells for a liquid-based preparation (Solomon *et al.*, 2002) and recommends assessing the cellularity by counting ten consecutive high-power fields (40X) along a diameter that includes the centre of the preparation. As a guide, 5,000 cells equates to an average of 3.8 cells per field (40X) with ThinPrep and 9.0 per field (40X) with SurePath. Details of these calculations and illustrations can be found in “The Bethesda System for Reporting Cervical Cytology: Definitions, Criteria and Explanatory Notes” (Solomon & Nayer 2004). While 5,000 is suggested as minimum cellularity, (Geyer *et al.*, 2000; Studeman *et al.*, 2003). Solomon *et al.* concluded that “additional studies relating sensitivity to cell number would be useful for all preparation types.” In the UK, although liquid-based cytology reduced the number of samples assessed as inadequate in the LBC pilot sites, as yet there are no agreed criteria for determining the adequacy of the cell sample (National Institute for Clinical Excellence (NICE), 2003).

Obscuration and other criteria

Other reasons for not being able to interpret smears are identical to those presented in TBS 1991 (Luff & et al, 1992). Specimens with >75% obscured cells (by blood, inflammation or air-drying) and smears which arrive broken or without appropriate patient identification must be considered unsatisfactory.

Transformation zone sampling

Although cross-sectional studies have consistently demonstrated a higher percentage of cytological abnormalities in conventional smears with evidence of transformation zone (TZ) sampling than in those without (Elias *et al.*, 1983; Vooijs *et al.*, 1985; Killough *et al.*, 1988; Boon & Suurmeijer, 1993), longitudinal studies have not shown an increased risk of high-grade lesions or cancer in women with smears lacking TZ sampling (Mitchell & Medley, 1991; Bos *et al.*, 2001;

Siebers *et al.*, 2003). For these reasons, absence of evidence of TZ sampling should not be used as the sole criterion for considering a smear to be unsatisfactory.

Clinical relevance of the quality judgment

While considering the adequacy of the smear, cytologists and smear-takers should be mindful of cervical cancer audits that have revealed smears preceding invasive cancer that might better have been reported as inadequate (Wilson & Johnson, 1992; DeMay, 1996; Wilson *et al.*, 1999). Furthermore, women with unsatisfactory smears have been shown to have a higher risk of an eventual diagnosis of high-grade abnormalities compared with those with negative smears (Ransdell *et al.*, 1997; Nygard *et al.*, 2004).

Cellular preparations are not labelled “unsatisfactory”, when epithelial cell abnormalities are discovered, even when the sample is of poor quality (Luff *et al.*, 1992).

Criteria for adequacy in opportunistic versus organised programmes

Criteria for adequacy of a cell sample will depend on whether the decision to repeat the test is made by the laboratory, as in the organised UK system, or whether the decision is made by the smear-taker. For example, the UK system discourages comments about obscuring exudate unless this is used as an explanation for an inadequate smear for which a repeat is recommended. TBS allows “quality indicator comments” about TZ sampling and exudate in slides formerly categorised as “satisfactory but limited by” with the assumption that a test may be repeated if deemed clinically necessary (Davey *et al.*, 2002).

Criteria for adequacy will also depend on the routine screening interval and whether or not women are discouraged from having screening tests at less than the recommended interval.

Recommendation

As a minimum, TBS criteria for conventional smears and LBC should be used, and if a specimen is judged to be unsatisfactory, the reason for the quality judgment should be provided on the cytology report.

Women with an unsatisfactory smear should be invited for a new smear, which must be monitored. Women should be contacted again if a new smear is not taken in due time.

Evidence of transformation zone sampling should be recorded although this is not a requirement on its own for a satisfactory sample.

3.6 Automated cytological screening

3.6.1 Description of automated screening devices

Two commercial systems were extensively studied in the 1990s: PAPNET (Neuromedical Systems Inc. (NSI), Suffern, New York, USA) and the AUTOPAP System (NeoPath Inc., Redmond, Washington, USA).

PAPNET includes neural network software and traditional imaging technology. It selects 128 of the most suspicious fields in conventional Pap smears and presents them on a PC monitor.

A cytologist interprets the images on the screen and decides to carry out manual screening when abnormalities are recognised or suspected

The Food and Drug Administration (FDA) of the United States has approved PAPNET for quality control of slides interpreted as negative after conventional screening. NSI has recently declared bankruptcy. TriPath Imaging Inc. (formerly AutoCyte, Burlington, NC, USA) has acquired the intellectual property of PAPNET.

AUTOPAP is a computerised scanning device designed for algorithmic classification of conventional Pap smears. It designates a score based on the likelihood that the slide contains an abnormality. AUTOPAP selects a predetermined proportion of slides that need further manual screening. The FDA has approved AUTOPAP for quality control and for primary screening (Dunton, 2000).

In the meantime, newer devices that target LBC smears are emerging, for instance: FOCAL POINT (TriPath Imaging Inc.) and ThinPrep IMAGER (Cytoc, Boxborough, MA, USA).

3.6.2 Rationale for automated screening

Automation-assisted screening aims to increase sensitivity and specificity, e.g., by finding small atypical cells, known to be very difficult to detect in conventional screening. These include both squamous and glandular cells. Screening performance should increase by excluding part of the slides from manual screening or by adding the most atypical cells to images or to fields examined under the microscope. By enhancing the effectiveness of slide processing, automation is expected to allow more slides to be screened by the same number of staff. This would be an advantage, because there is a severe shortage of cytotechnicians in many countries. Some automated devices are capable of processing either conventional or liquid-based smears and can therefore be used in different kinds of screening programmes.

The aims of automated screening are: (1) to increase sensitivity and specificity of cytological screening; (2) to decrease the workload of cytotechnicians and cytopathologists; (3) to decrease the cost of screening programmes; and (4) to decrease the incidence and mortality of cervical cancer.

3.6.3 Evaluation of performance

Several published articles have evaluated the performance of automation-assisted screening (Fahey *et al.*, 1995; Wilbur *et al.*, 1996; Kok & Boon, 1996; Michelow *et al.*, 1997; Koss *et al.*, 1997; Halford *et al.*, 1999; Doornewaard *et al.*, 1999; PRISMATIC Project Management Team, 1999; Kok *et al.*, 2000; Duggan, 2000; Bergeron *et al.*, 2000). In general, they show better test sensitivity with at least the same specificity as conventional screening. Most of these articles have been retrospective (quality control) and/or have involved relatively small numbers of smears. The Prismatic study (PRISMATIC Project Management Team, 1999) showed equal sensitivity but better specificity for automated screening as well as better productivity (faster screening) in a prospective study with 21,700 smears. Ronco *et al.* (2003) also found substantially reduced interpretation time and good agreement in classification with map-guided vs. conventional interpretation. Only two randomised, prospective public health trials in a primary screening setting have been published thus far. The first of these studies reported higher detection rates of CIN3 and of more serious cases, specifically in situ-carcinoma and invasive carcinoma (Kok & Boon, 1996).

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The second study, in an organised screening setting and involving several cytological laboratories, did not confirm this result (Table 4) (Nieminen *et al.*, 2003). Instead, using histologically confirmed CIN+ as an endpoint, the latter study showed sensitivity almost equal to that of the traditional cytological screening technique. Specificity and positive predictive value were at an equal level in automation-assisted screening when compared to manual conventional screening. These parameters were reported only in the latter study (Table 5).

Table 4. The number (N) and proportion (/1000) of screenees by histology and study arm. Odds ratios (OR) with 95% confidence intervals (CI) of histologically verified cervical lesions in the PapNet arm in comparison with the conventional screening arm (logistic regression). Data from Kok & Boon, 1996 (a) and Nieminen *et al.*, 2005 (b).

a.						
Histology	Papnet arm (total 65 527)		Conventional arm (total 25 767)		OR	p
	N	/1000	N	/1000		
Invasive cancer	42	0.64	8	0.31	2.16	p < 0.05
In situ carcinoma	79	1.20	18	0,68	1.76	p < 0.05
CIN3	124	1.89	44	1,70	1.11	n.s
b.						
Histology	Papnet arm (total 110 191)		Conventional arm (total 220 254)		OR	CI
	N	/1000	N	/1000		
Invasive cancer	14	0.13	25	0.11	1.12	0.59-2.13
CIN 3	138	1.3	295	1.3	0.94	0.76-1.14
CIN2	132	1.2	303	1.4	0.87	0.71-1.07
CIN1	134	1.2	250	1.1	1.07	0.87-1.32
Normal and other	109,773	996	219,381	996	1.00	reference

Table 5. Specificity of the Papnet and conventional Pap smear test with cut off levels of ASCUS+ and LSIL+ for invasive cancer and for an outcome of CIN3+ in primary screening setting. Total N=330,445 women (Nieminen *et al.*, 2005).

	Negative histology	Negative Pap smear	Specificity %
Cytological threshold: ASCUS+			
Papnet	110 039	100 894	91.7
Conventional	219 934	202 256	91.9
Cytological threshold: LSIL+			
Papnet	110 039	109 394	99.4
Conventional	219 934	218 598	99.4

Technological development is very rapid in this area. New technology is emerging and some of the older devices are no longer commercially available.

When implementing automated-assisted methods, it is necessary to carefully ascertain and evaluate the performance of the method in primary (public health) screening up to the final invasive end-points in randomised prospective studies. It is therefore important to organise the trials in such a way that the investigated technology can be used for several years in the trial, irrespective of its current or future commercial availability (Nieminen *et al.*, 2005).

3.6.4 Conclusion

The few studies applying a robust design have shown that automation-assisted screening performs equally well compared to conventional screening in an organised, quality-controlled setting. There is no current evidence of increased sensitivity and specificity for relevant pre-invasive lesions with computer-assisted cytology. The advantage depends on increased productivity and must be compared with the costs of the equipment.

Currently, a new generation of screening devices targeting essentially liquid-based cytology smears is undergoing evaluation. The new models should also be tested in prospective randomised trials before adopting them for use in routine screening.

3.7 Colposcopy

3.7.1 Description

The colposcope is an optical instrument that allows observation of the cervix and vagina under optimal illumination at magnification between 6X and 40X. The aim of colposcopy is to allow the trained colposcopist to identify a premalignant disease of the cervix.

In a premenopausal woman, examination can be carried out at any phase of the menstrual cycle, but ideally it is performed during the estrogenic phase. In patients with atrophic cervical epithelium, the assessment may be inconclusive in which case examination should be repeated after the woman has had a course of parenteral or vaginal oestrogen. Ideally, it is better not to perform colposcopy after a cervical smear has been taken, because scraping of the cervix may cause bleeding and make it more difficult to assess the epithelium.

After macroscopic examination of the vulva, an appropriate vaginal speculum is inserted taking care not to injure the cervix. Examination should commence at a low magnification after rinsing the cervix with normal saline and removing any excess cervical mucous. A green filter may be used at this stage of the examination to facilitate assessment of the sub-epithelial capillaries. A 3% or 5% acetic acid solution is then applied to the cervix. An aceto-white reaction occurs when the squamous epithelium is abnormal. Acetic acid causes tissue oedema and superficial coagulation of intracellular proteins, thus reducing the transparency of the epithelium. Unfortunately, not all the areas of aceto-white epithelium indicate the presence of premalignant disease, for example areas of immature metaplasia are aceto-white.

A further technique is to apply Schiller's iodine or Lugol's iodine. Normal squamous epithelium is rich in glycogen and stains dark brown with iodine, whereas premalignant squamous epithelium is deficient in glycogen and is non-staining. This is a good method for demarcating abnormal areas when the cervix is actually being treated. However, not all non-staining areas represent premalignant disease.

Complete colposcopic examination requires observation of the original squamous epithelium, the entire transformation zone, the squamo-columnar junction and as much of the columnar epithelium of the cervix as possible. Locating the squamo-columnar junction is a key procedure in colposcopic assessment. If the squamo-columnar junction is not visible, or only partially visible, i.e. if the upper (endocervical) limit of the normal or atypical squamous epithelium is not visible completely, then the examination is unsatisfactory.

As the speculum is being withdrawn, the vagina should be inspected.

Once the colposcopic examination is completed, it is essential that all observations must be entered on a structured colposcopy chart. The chart should show the situation of the squamo-columnar junction and clearly define the topography and nature of the different lesions, as well as biopsy sites (see Chapters 2 and 6).

3.7.2 Accuracy of colposcopy

Colposcopy requires long-term experience to acquire expertise in colposcopic pattern recognition. The expert colposcopist may be able to predict the histological diagnosis quite accurately, but in general, the colpo-histological correlation is only moderate. Even after several years of colposcopic practice, inter-observer and intra-observer variations of colposcopic interpretations may not reach Kappa values greater than 0.50 (Hopman *et al.*, 1995; Etherington *et al.*, 1997).

Unbiased assessment of the accuracy of a test requires the independent verification with a gold standard, which usually relies on histology. This is particularly difficult for colposcopy since the choice of the biopsy site depends on colposcopy itself. Because of this intrinsic dependency, sensitivity estimates for colposcopy are inflated. Colposcopically negative cases are very often considered as truly negative without histological confirmation. Moreover, in case of glandular cervical lesions or endo-cervical location of the SCJ, colposcopy may be falsely negative.

In a meta-analysis including 9 studies, the sensitivity and specificity of colposcopy for detecting CIN2+, was estimated to be 96% and 48% (Mitchell *et al.*, 1998). However, most studies included in the meta-analysis suffered from the aforementioned bias. In one particular study, conducted in China, cervical biopsies were taken not only from colposcopically suspect areas but also from the four quadrants of the transformation zone in colposcopically negative cases (Pretorius *et al.*, 2001; Pretorius *et al.*, 2004; Pretorius *et al.*, 2006). This design allows for a more unbiased assessment of colposcopic accuracy. The sensitivity of colposcopy-directed biopsy for CIN2+ in women with satisfactory colposcopy was 57% (Pretorius *et al.*, 2004). In the ALTS study also, immediate baseline colposcopy identified only 54% or 56% of cumulative CIN3+ cases diagnosed over 2 years in women with ASCUS or LSIL, respectively (ASCUS-LSIL Triage Study Group, 2003a; ASCUS-LSIL Triage Study Group, 2003b).

3.7.3 Conclusions

Because of its low specificity, colposcopy is not recommended as a screening tool.

Recent data have demonstrated that the sensitivity of colposcopy for existing or incipient high-grade cervical intra-epithelial neoplasia is substantially lower than usually assumed.

Colposcopy is an essential triage method for the management of women with abnormal cytology. For a more extensive description of indications and best practice recommendations, see Chapter 6.

3.8 HPV DNA detection

3.8.1 Introduction

The recognition of the strong causal relationship between persistent infection of the genital tract with high-risk human papillomavirus (HPV) types and occurrence of cervical cancer (Bosch *et al.*, 2002; IARC, 2005) has resulted in the development of a series of HPV DNA or RNA detection systems. Detection of high-risk HPV DNA is considered to be potentially useful in three clinical applications: 1) as a primary screening test, solely or in combination with a Pap smear to detect cervical cancer precursors; 2) as a triage test to select those women with minor cytological lesions in a Pap smear who require referral for diagnosis and treatment and 3) as a follow-up test to predict cure or failure of treatment with local ablative or excisional therapy in women treated for high-grade intra-epithelial lesions (Cuzick *et al.*, 1999b).

For primary screening, previous reviews conducted by Lorincz and Richart (2003), Franco (2003), the IARC (2005) have been updated and the results have been pooled in a formal meta-analysis. (Koliopoulos *et al.*, 2006; Arbyn *et al.*, 2006). This meta-analysis addressed only cross-sectional test accuracy for high grade CIN. Results of greater public health relevance are not yet available from the large randomised trials that are currently being conducted in several European countries (Davies *et al.*, 2006).

Two reviews comparing cytological and virological triage of minor cytological lesions and post-treatment follow-up, conducted within the framework of the European Cervical Cancer Screening Network, were published earlier (Paraskevaidis *et al.*, 2004; Arbyn *et al.*, 2004a & b; Arbyn *et al.*, 2005).

3.8.2 HPV nucleic acid detection systems

Several major HPV DNA tests are described below that can be used in high-throughput settings.

3.8.2.1 Hybrid Capture 2

The Hybrid Capture 2 assay (Digene Corp., Gaithersburg, Maryland, USA) is a commercially available HPV test that uses RNA probes and a sensitive detection of the captured DNA/ RNA hybrids,

but which does not involve target DNA amplification (Lorincz, 1997). Detection of HPV DNA yields a light signal, the intensity of which is related to the viral load.

This test has the advantage of availability in a standardised kit format that can be used by most laboratories. The test has been used in several cross-sectional and longitudinal studies and has been shown to have a high sensitivity for detection of high-grade CIN and cancer (Lorincz, 1997; Sherman *et al.*, 2003; IARC, 2005). A disadvantage is that the test does not permit determination of the HPV type in the sample. It only indicates the presence or absence of oncogenic HPV because the test hybridises with a mixture of probes. There is a probe cocktail B, targeting 13 oncogenic HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68), and another probe cocktail A, targeting 5 benign HPV types (6, 11, 42, 43 and 44). Apart from the HPV types that the test is designed to detect, the test has also been found to detect additional HPV types that cross-hybridise with the probe mix (Peyton *et al.*, 1998; Vernon *et al.*, 2000; Peyton *et al.*, 2001). Cross contamination with other high-risk types can be considered to be beneficial, but take-up of low-risk types clearly decreases specificity (Castle *et al.*, 2002b).

The Hybrid Capture 2 assay is the only commercially available HPV DNA detection test that is approved by the FDA for cervical cancer screening in combination with cytology after the age of 30.

3.8.2.2 General primer PCR based on the primer pair GP5+/GP6+

The GP5+/6+ polymerase chain reaction system is an extended version of the GP5/6 PCR, which uses a simple pair of consensus primers. The GP5+/6+ test amplifies a 140 bp region in the L1 gene and has shown a high sensitivity and specificity for prediction of high-grade CIN (Jacobs, 1997). The test has been developed to a simple, rapid enzyme immunoassay-PCR (EIA-PCR) format that is suitable for processing large amounts of samples. An international validation study that was performed before the start of a primary HPV screening trial in Sweden found limited interlaboratory variation (Kappa statistics of at worst 0.88, at best 1.0) (Elfgren, 2003). Comparison of reproducibility between different HPV tests in the same study found lower agreement, implying that inter-method variability was considerably greater than intra-method inter-laboratory variation.

3.8.2.3 General primer MY09/11 system

This PCR test amplifies a 450 bp region in the L1 gene. The test is presently used with an improved primer design (2 sets of non-degenerated PGMY09/11 primers), that has been found to have better consistency and better sensitivity for a broad range of HPV types than the original MY09/11 primers (Gravitt *et al.*, 1998).

There are several methodological studies that have compared this test to either the Hybrid Capture or the GP5+/GP6+ PCR system. The sensitivity for detection of cervical neoplasia appears to be about the same, but there is a disturbing amount of discrepant results. Qu *et al.* (1977) found an overall agreement of 0.79 (kappa statistic) and Elfgren *et al.* (2003) reported a kappa statistic of 0.68 when comparing MY09/11 and GP5+/GP6+. Peyton *et al.* (1998) found a kappa of 0.58 when comparing MY09/11 and Hybrid Capture.

Part of the discrepancies, but only part, can be explained by differential sensitivities for certain HPV types (Jacobs *et al.*, 1999; Konya *et al.*, 2000). For instance, the MY09/11 primers are less sensitive for amplification of HPV 35 and GP5+/GP6+ are less sensitive for amplifying HPV 53 and 61 (van der Graaf *et al.*, 2002).

There is also a striking difference in the amount of samples that are simultaneously positive for several HPV types by the different systems, with MY09/11 assays reporting many more multiple HPV positivities (Kornegay *et al.*, 2001).

The difference results from the fact that GP uses one consensus primer pair that will selectively bind with highest affinity in the first amplification round to one HPV type in a mixture, whereas a mix of primers allows binding of different types with comparable affinity at the same time.

3.8.2.4 SPF10 PCR

The SPF10 PCR amplifies a DNA sequence of only 65 bp from a highly conserved region of the viral L1 gene (Kleter *et al.*, 1998; Kleter *et al.*, 1999). Given the shortage of the amplicon, the analytical sensitivity is very high, but for the same reason type discrimination is complex (Iftner & Villa, 2003). SPF10 amplification was shown to be useful for HPV DNA testing in archived smears, in which parts of the viral genome can be damaged. It is also used in the LiPA HPV typing system (see below).

3.8.2.5 Amplicor Human Papillomavirus Test

The Amplicor Human Papillomavirus Test (Roche Molecular Diagnostics) is the first commercially available PCR kit. It uses a non-degenerate set of primers that targets a short 170 bp fragment of the L1 gene of the same 13 high-risk HPV types included in the Hybrid Capture 2 assay. The kit employs the TaqGold DNA polymerase, which minimises non-specific amplification and increases sensitivity (Iftner & Villa, 2003). Since only a short DNA sequence is targeted, analytical sensitivity is higher than systems targeting longer fragments.

3.8.2.6 Real time PCR

In real-time PCR, fluoresceine bound to the primer, is released by the 5'-exonuclease activity of the Taq DNA polymerase. The intensity of fluorescence is directly proportional to the amount of amplified DNA and is measured in real-time by an automated fluorometer. It therefore allows a precise estimate of the quantity of target DNA that is present in a sample (Swan *et al.*, 1997; Josefsson *et al.*, 1999). RT PCR can also be applied in multiplex format, where presence of and viral load of multiple HPV types can be assessed simultaneously and with control of the amount of input DNA (Tucker *et al.*, 2001; Moberg *et al.*, 2003).

3.8.2.7 HPV DNA typing methods

After PCR amplification, distinction of HPV types can be achieved by reverse hybridisation with type-specific probes using a variety of formats, such as line strip assays and micro-titre plates (Iftner & Villa, 2003). Van den Brulle *et al.* (2002) developed a reverse line blot analysis enabling rapid and high-throughput identification of 37 human papillomavirus genotypes after GP5+/GP6+ amplification.

The INNO-LiPA HPV genotyping kit (Innogenetics, Gent, Belgium) is a commercially available line probe assay allowing detection of 25 HPV genotypes (6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 52, 53, 56, 58, 59, 66, 68, 70, 73, 74) after SPF-10 PCR amplification (Quint *et al.*, 2001; van Doorn *et al.*, 2002). The Linear Array HPV Genotyping Test (Roche Diagnostics, Indianapolis, USA) is another commercially available HPV genotyping system, which allows determination of 37 HPV types (6, 11, 16, 18, 26, 31, 33, 34, 35, 39, 40, 51, 52, 53, 56, 58, 59, 61, 62, 64, 66, 67, 72, 73, 81, 82, 83, 84, 89, IS39, CP108) using oligonucleotide probes after PGMY09/11 PCR amplification. Identification of types is also possible by using PCR with type-specific primers targeting specific sequences of the viral E genes (Moberg *et al.*, 2003; Moberg *et al.*, 2004).

In a reproducibility study in which three HPV typing methods were evaluated on samples with one HPV type determined by reversed line blot (RLB) hybridisation, kappa was 0.87 for RLB vs Inno-LiPA, 0.94 for INNO-LiPA vs. Amplisense (Nuclear Laser Medicine; (Carcheri *et al.*, 2003) and 0.82 for RLB vs Amplisense. Agreement was substantially lower for the presence of multiple infections (Gillio-Tos *et al.*, 2006).

3.8.2.8 DNA micro-array chips

In the DNA microarray detection system developed by Biomedlab Company (Seoul, South-Korea) type specific oligonucleotide probes and a control probe for beta-globine DNA are fixed to a slide. The sample is first submitted to PCR amplification in the presence of fluoresceinated nucleotides. The amplicons are subsequently hybridised on the slide and laser-scanned (Kim *et al.*, 2003).

Another DNA-chip system is the *PapilloCheck® HPV screening* (Greiner-Bio-One, Frickenhausen, Germany) using amplification in a fragment of the E1 gene. It is a DNA-array based diagnostic tool for the simultaneous detection and genotyping of 24 different HPV types (including 6 low-risk types [6, 11, 40, 42, 43, 44] and 18 high-risk or putative high-risk types [16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, 82]) using automatic scanning on a slide.

Otherwise, Genomica (Coslada-Madrid, Spain), developed *Clinical Arrays HPV®*, is an innovative low-density array tube with the array placed on the bottom of a small tube. It can detect 35 HPV genotypes (including 24 types included in PapilloCheck but also 5 low-risk types [54, 61, 70, 72 and 81], 1 intermediate high-risk type [26] and 6 indeterminate types [62, 71, 83, 84, 85 and 89]) using amplification of 450 bp in L1 region, with an automatic colorimetric detection. The last two systems have amplification controls.

Luminex microarray technology is an innovative method allowing high-throughput simultaneous identification and quantification of large series of HPV types (Wallace *et al.*, 2005; Schmitt *et al.*, 2006). In the first step, HPV DNA is amplified using a PCR with, for instance, PGMY09/11 of GP5+6+ PCR. Genotyping is based on hybridisation with type-specific oligonucleotide probes coupled to suspended polystyrene beads which are dyed with various ratios of two spectrally distinct fluorophores. Subsequently, hybridised beads are injected in a Luminex analyzer which recognises the spectral signatures. This liquid bead microarray technique showed excellent analytical sensitivity, specificity and reproducibility with a validated RLB-based genotyping system (Schmitt *et al.*, 2006).

3.8.2.9 Detection of viral oncogene transcripts

Viral mRNA can be detected using (nested) real-time-PCR (nRT-PCR) or nucleic acid sequence-based amplification assay (NASBA) (Smits *et al.*, 1995; Sotlar *et al.*, 1998). Presence of viral mRNA transcripts coding for the oncoproteins E6 and E7 from high-risk papilloma viruses might be a more specific predictor of progressive infection than simple presence of HPV DNA (Nakagawa *et al.*, 2000; Cuschieri *et al.*, 2004). A commercial kit exists (PreTect HPV-Proofer, NorChip AS, Kokkastua, Norway) for detection of E6 mRNA from HPV 16 and E7 mRNA from the HPV types 18, 31, 33 and 45.

Viral integration in the human genome, often occurring in the E2 region, results in interruption of HPV DNA and enhanced transcription of the E6-E7 sequence, a condition that pre-determinates to neoplastic transformation (zur Hausen, 2002). Under such conditions, tests based on L1 DNA detection can be negative, whereas E6 or E7 mRNA or DNA type specific tests will be positive.

Molden *et al.*, found rates of HPV-Proofer positivity and presence of HPV DNA (measured with GP5/6+ consensus PCR and type specific PCR) that increased with severity of cytological or histo-

logical cervical abnormality (Molden *et al.*, 2005b). Nevertheless, lower proportions of mRNA-positive results were observed in normal cases, ASCUS, and LSIL, which could be interpreted as a possible increase in specificity compared to HPV DNA testing.

3.8.2.10 Conclusion

In summary, HPV testing has advanced enormously in performance, but the major available tests conceivable for use in clinical practice have been evaluated to different extents.

It is clear that continuous monitoring of quality is necessary for studies and clinical applications. Further improvements and standardisation to reduce discrepancies is desirable for: the DNA (or RNA) extraction step before PCR, the choice of different HPV genes (L1, E6, E7 or E1) for the oligonucleotide position, and the choice of protocols and machines (for PCR, hybridisation and detection). With regard to general primer PCR-based tests, the fact that some systems are not yet commercially available is currently a significant drawback for routine use.

Analytical or chemical sensitivity (expressed as minimum number of HPV genomes/ml) should be distinguished from clinical sensitivity (ability to detect lesions). Ultra-sensitive PCR systems do not necessarily increase the screening or triage sensitivity compared to established systems (such as MY09/MY11 or GP5+/GP6+ PCR or HC2) because detection of a very low viral load is not associated with an increased risk for CIN (Ylitalo *et al.*, 2000; Josefsson *et al.*, 2000; Snijders *et al.*, 2003). The new generation of ultra-sensitive genotyping systems is certainly useful in epidemiological studies and vaccination trials. Nevertheless, application of these systems in routine practice warrants caution and should be discouraged because of the lack of clinical validation (Meijer *et al.*, 2006).

Study protocols are needed to compare different HPV detection methods and to establish test performance equivalency.

In the future, international standard reagents for calibration and reproducible analytical assessment of HPV test assays, and also robust quality control procedures will be needed to assure the correct use of HPV tests in clinical practice and particularly for potential primary HPV-based screening programmes for cervical cancer (Quint *et al.*, 2006; Pagliusi *et al.*, 2006).

3.8.3 Use of HPV testing in primary screening

3.8.3.1 Cross-sectional accuracy

Absolute accuracy

A recent meta-analysis, which was also registered as a Cochrane review, compared the test performance of HPV DNA testing in primary screening (Koliopoulos *et al.*, 2006; Arbyn *et al.*, 2006). The meta-analysis included 24 studies in which a Pap smear was taken and at the same time a sample was collected for HPV DNA detection. The high-risk probe of the Hybrid Capture 2 (HC2) was employed 17 studies, and in the 7 others a PCR system was applied. The aim was to find underlying CIN2, CIN3 or cervical cancer. Verification consisted in colposcopy and histological examination of punch biopsies from colposcopically suspect areas, excision biopsies or endocervical curettage. In most cases, the histological result and, if absent, a negative result of a satisfactory colposcopy examination was accepted as gold standard diagnosis. In 10 studies, gold standard verification was limited to women with at least one positive screening test result; in 8 studies, a random sample of screen-negative women was also referred for colposcopy, allowing adjustment for verification bias. In the other 6 studies, all women underwent colposcopy. The meta-analysis of the relative sensi-

tivity included the preliminary baseline data from 2 published randomised controlled trials in which women were randomised to cytology or HPV testing (Sankaranarayanan *et al.*, 2005; Kotaniemi-Talonen *et al.*, 2005). The principle results describing the pooled estimate of the sensitivity and the specificity of HPV testing, the range of minimal and maximum values, the test positivity, and the prevalence of precancer (for the outcomes CIN2+ and CIN3+) are summarised in Table 6.

Overall, the sensitivity of HC2 for detecting underlying high grade intra-epithelial neoplasia was 89.5% (95% CI: 85.1-93.1%) but varied over a large range between 50% (Sankaranarayanan *et al.*, 2004) and 100% (Clavel *et al.*, 2001). The observed sensitivity of HC2 was extremely low in the three cross-sectional studies conducted in India: 50%, 70% and 80%, respectively (Sankaranarayanan *et al.*, 2004), and was also lower than average in other developing countries (81% in Zimbabwe (Blumenthal *et al.*, 2001), 83% in Brazil (Sarian *et al.*, 2005), 88% in South-Africa (Kuhn *et al.*, 2000). However, the sensitivity for CIN2+ was consistently high in six studies conducted in Europe and North-America: pooled estimate of 97.9% (95% CI: 95.9-99.9%; *p* for inter-study heterogeneity = 0.22) (Ratnam *et al.*, 2000; Clavel *et al.*, 2001; Coste *et al.*, 2003; Petry *et al.*, 2003; Cuzick *et al.*, 2003; Bigras & De Marval, 2005).

The overall pooled specificity of HC2 in excluding high-grade cervical pre-cancer was 87.5% (95% CI: 85.0-89.9%; range: 61-95%). In North-America and Europe, the pooled specificity was higher: 91.3% (95% CI: 89.5-93.1%; range: 85-95%).

In seven studies, a PCR system was used for detecting HPV DNA sequences (Cuzick *et al.*, 1995; Cuzick *et al.*, 1999a; Schneider *et al.*, 2000; Oh *et al.*, 2001; Paraskevaidis *et al.*, 2001b; Kulasingam *et al.*, 2002; Agorastos *et al.*, 2005). Its pooled sensitivity for CIN2+ (80.9%; 95%: 70.0-91.7%) was lower, but its pooled specificity (94.7%; 95%: 92.5-96.9%) was higher compared to the HC2 assay. Nevertheless, given the use of different primers and detection of amplified sequences, this conclusion cannot be generalised. For instance: the sensitivity was 95% in a German study in which GP5+/GP6+ primers were used followed by hybridisation with a cocktail of oligonucleotides of 14 high-risk HPV types (Schneider *et al.*, 2000) and only 64% in a British study in which the PCR/Sharp assay was used (MY09/MY11 primers, hybridisation with 10 high-risk types) (Cuzick *et al.*, 1999a).

The sensitivity and specificity of the *combination of the HC2 assay and cytology*, considering ASCUS as cutoff for positivity, for detecting CIN2+, pooled from the 6 North-American and European studies, were 99.2% (95% CI: 97.4-100%) and 87.3% (95% CI: 84.2-90.4%) respectively. Overall, 14.5% (95% CI: 11.0-18.1%) of screened women showed a positive result for at least one test.

From a multi-variate regression it was concluded that geographical differences, probably due to the differences in quality of the gold standard, explained most of the interstudy heterogeneity. Completeness of verification was not significantly associated with inter-study heterogeneity, suggesting minor impact of verification bias, as could be expected, given the high sensitivity of the HPV test.

Relative accuracy

The relative sensitivity and specificity of HPV testing compared to cytology, considering ASCUS or LSIL as a cut-off, is summarised in Table 7. Overall, the sensitivity of HC2 was 23% higher (95% CI: 13-23%). In one randomised trial (India), the detection rate of CIN2+ was lower in the HPV screened arm compared to the cytology arm. In all other studies, the sensitivity of HC2 was higher, varying from +1% to +115%. The pooled specificity of HC2 was overall 6% lower than cytology (ratio: 0.94; 95% CI: 0.92-0.96; range: 0.67-1.10). PCR was also more sensitive than cytology for detecting CIN2+ (ratio: 1.25; 95% CI: 0.95-1.63) but this difference was not significant due to the huge heterogeneity among studies. The highest values of relative sensitivity were observed in Germany (1.63 (Schneider *et al.*, 2000) and 2.15 (Petry *et al.*, 2003)), likely due to the poor sensitivity of cytology.

The combination of cytology with HC2 was 45% (95% CI: 31-60%) and 39% (95% CI: 11-73%) higher, respectively, for the detection of respectively CIN2+ or CIN 3+ than cytology alone (at cut-off ASCUS+), whereas the specificity was 7% lower (95% CI: 6-8%). Adding a Pap smear to the HC2 test and considering ASCUS or worse as a positive cytological result increased the sensitivity of HC2 for CIN2+ or CIN3+ by 7% and 4%, respectively, but resulted in a loss in specificity of 5% (95% CI: 4-6%) and 7% (95% CI: 5-9%).

Reproducibility

HPV testing with a validated test is objective and highly reproducible. It lacks the interlaboratory/interobserver variability of cervical cytology. Castle found good agreement when retesting frozen samples from a Costa Rican population with the HC2 assay (un-weighted kappa of 0.72) (Castle *et al.*, 2002a). High agreement in HC2 results was also found in a quality assurance exercise in seven Italian laboratories (overall kappa=0.95 with ThinPrep samples and 0.96 with STM samples) (Carozzi *et al.*, 2005).

3.8.3.2 Age groups to be targeted and screening intervals for HPV screening programmes

In Chapter 2 recommendations on the age groups to be targeted with cytological screening are provided mainly on the basis of age-specific incidence of cervical cancer. The same considerations apply to screening by HPV testing. To define the most appropriate target age group and frequency of HPV screening, a thorough knowledge of the epidemiology and natural history of HPV infections is needed as well.

Several European studies have evaluated the prevalence of HPV infection in various age groups (Hagmar *et al.*, 1995; Chua *et al.*, 1996; Kjellberg *et al.*, 1998; Cuzick *et al.*, 1999a; Forslund & Antonsson, 2000; Jacobs *et al.*, 2000; de Sanjose *et al.*, 2003; Cuzick *et al.*, 2003; Ronco *et al.*, 2005). The prevalence of oncogenic HPV types is higher in younger women and declines with increasing age. This may be attributed to the transient character of most infections at young age and to changes in sexual behaviour, resulting in decreasing rates of acquisition in older women. However there is some variability in the general and age-specific HPV prevalence throughout Europe: for example, prevalence at any age, including young women, is low in Spain (de Sanjosé *et al.*, 2003).

In a study performed in Costa Rica, persistence of HPV infections was observed to increase with age (Castle *et al.*, 2005a). In a cohort of teenagers, in the UK, who had recently become sexually active, surveillance at short intervals revealed a high frequency of HPV infection (3-year cumulative incidence of 44%); high grade CIN subsequently developed in 5% of the HPV positives (Woodman *et al.*, 2001)

The HPV prevalence in young women, combined with low cancer incidence at this age, suggests poor efficiency of HPV testing. HPV screening at that age has a low specificity and will result in treatment of many transient lesions. Cuzick showed that the specificity of HPV screening for excluding presence of CIN2+ was on average 7% higher in women of 35 years or older compared to younger subjects (Cuzick *et al.*, 2006). Although there is no clear breakpoint above which the presence of hrHPV is associated with a higher predictive value for future progressive disease, 30 years seems to be a plausible age to start HPV screening in Europe (WHO 2006).

Table 6. Summary of meta-analyses on the test performance of HPV DNA testing using HC2 or PCR in primary cervical cancer screening. Sensitivity and specificity (pooled estimate, p-value for inter-study heterogeneity and range (minimum and maximum observed value) to detect histologically confirmed CIN2+ or CIN3+, pooled test positivity rate, and prevalence of CIN¹.

Test	Test cut-off	Outcome	Studies	Test sensitivity			Test specificity			T+ rate	Prevalence
				pooled estimate (95% CI)	p	Range (%)	pooled estimate (95% CI)	p	Range (%)		
HC2	1pg/mL	CIN2+	16	89.5 (85.1-93.1)	0.00	50-100	87.5 (85.0-89.9)	0.00	61-95	14.2 (11.3-17.1)	2.3 (1.8-2.8)
		CIN2+	6**	97.9 (95.9-99.9)	0.22	84-100	91.3 (89.5-93.1)	0.00	85-95	9.9 (7.8-12.0)	1.2 (0.8-1.5)
		CIN3+	8/7	89.0 (82.5-95.5)	0.00	62-98	90.8 (88.4-93.2)	0.00	84-95		1.0 (0.7-1.2)
PCR	+signal	CIN2+	6	80.9 (70.0-91.7)	0.01	64-95	94.7 (92.5-96.9)	0.00	79-99	7.3 (4.4-10.3)	2.5 (1.3-3.6)
HC2 & cytology	1pg/mL or ASCUS+	CIN2+	6***	99.2 (97.4-100)	0.95	98-100	87.3 (87.3-90.4)	0.00	69-94	14.5 (11.0-18.1)	1.2 (0.8-1.5)

* If multiple visits per patient were documented, values from the visit near 6 months after treatment were chosen for pooling.

** Restricted to studies conducted in North-America or Europe.

*** After exclusion of studies conducted in India and Zimbabwe (Blumenthal *et al.*, 2001; Sankaranarayanan *et al.*, 2004).

Table 7. Relative accuracy of virological versus cytological screening or of combined screening versus testing with one test in order to find underlying CIN2 or CIN3 or worse¹.

Comparison	Outcome	Relative sensitivity		Range	Relative specificity		Range	#Studies
HC2 vs. cyto (ASCUS or LSIL+)	CIN2+	1.23	(1.13-1.33)	0.87-2.25	0.94	(0.92-0.96)	0.67-1.10	18/16*
PCR vs. cyto (ASCUS+)		1.25	(0.95-1.63)	0.75-3.57	0.99	(0.96-1.02)	0.86-1.08	6
HC2 vs. cyto (ASCUS+)	CIN3+	1.28	(1.12-1.47)	0.97-2.12	1.00	(0.99-1.01)	0.96-1.10	7
Cyto (ASC+) or HC2 vs. Cyto (ASCUS+)	CIN2+	1.45	(1.31-1.60)	1.06-2.30	0.93	(0.92-0.94)	0.89-0.96	9
Cyto (ASC+) or HC2 vs. Cyto (ASCUS+)	CIN3+	1.39	(1.11-1.73)	1.02-2.18	0.93	(0.92-0.94)	0.89-0.95	6
Cyto (ASCUS+) or HC2 vs. HC2+	CIN2+	1.07	(1.06-1.08)	1.02-1.37	0.95	(0.94-0.96)	0.81-0.99	9
Cyto (ASCUS+) or HC2 vs. HC2+	CIN3+	1.04	(1.03-1.04)	1.02-1.17	0.93	(0.91-0.95)	0.81-0.99	6

* The meta-analysis of relative sensitivity includes 2 RCTs, the meta-analysis of relative specificity does not include RCTs

¹ Adapted from Arbyn M., Sasieni P., Meijer C.J., Clavel C., Koliopoulos G., & Dillner J. (2006). Chapter 9: Clinical applications of HPV testing: A summary of meta-analyses. *Vaccine* **24 S3**: 78-89 with permission of Elsevier.

A high longitudinal negative predictive value was observed in women having a baseline negative Hybrid Capture 2 result (Sherman *et al.*, 2003; Lorincz & Richart, 2003; Kahn *et al.*, 2005). This suggests the possibility of applying prolonged screening intervals. The large randomised trials comparing HPV testing with cytology screening which are currently underway in Europe are expected to provide more clear answers to this issue (Davies *et al.*, 2006).

The high longitudinal negative predictive value of HPV testing also suggests the possibility of an anticipated stop of screening in HPV-negative women. Data on incidence of new infections in middle aged/older women are actually lacking. These would be essential for a rational choice of stopping age.

In conclusion, primary screening by HPV testing, if practiced, should not start below age 30 and will probably permit longer screening intervals. Rational HPV-based screening policies may be developed in the near future based on upcoming trial results. Meanwhile, more research is needed to determine the age-specific epidemiology of HPV infections in different populations.

3.8.3.3 Possible strategies to improve specificity of HPV testing for primary screening

Longitudinal studies of HPV infection show that infections normally clear within 1-2 years (Hildesheim *et al.*, 1994; van Doornum *et al.*, 1994; Ho *et al.*, 1995; Evander *et al.*, 1995). However, it is clear that the women who develop CIN or cancer are persistently positive for high-risk HPV DNA in repeated tests (Remmink *et al.*, 1995; Rozendaal *et al.*, 1996; Chua & Hjerpe, 1996; Cuzick *et al.*, 1999b; Forslund & Antonsson, 2000). Simply **repeating the HPV test** to identify persistent infections is therefore one possible method to increase the specificity of primary HPV screening. However, recent data from a cohort study in Costa Rica, have shown that one third of women with **persistent HPV 16 infection** developed CIN3 or cervical cancer within five years, whereas persistent low-risk HPV infections virtually never caused CIN3. (Schiffman *et al.*, 2005a).

As explained in Section 3.8.3.2, restriction of HPV screening to **older age-groups** yields higher specificity.

Another possible method to identify the women at highest risk is by testing for a **high amount of viral DNA, or "high viral load"** (Clavel *et al.*, 1999; Ylitalo *et al.*, 2000; Josefsson *et al.*, 2000). Restriction to samples that contain a substantial viral load, reduces the risk of contamination and increases the probability that a positive result does indeed reflect a true infection. For Hybrid Capture 2 higher specificity with a negligible decrease in sensitivity was observed in European trials when increasing the cut-off from 1 to 2 pg/ml (Cuzick *et al.*, 2003; Ronco *et al.*, 2006b). However, in a high-risk population in Costa Rica the optimal cut-off was at 1pg/ml (Schiffman *et al.*, 2000).

Finally, testing for **HPV integration**, detection of **mRNA coding for the oncogenes E6 or E7** from a limited set of high-risk HPV types, and immunostaining of overexpressed cell-cycle regulating proteins (for instance p16) appears to increase the probability that the HPV positive sample is derived from a sample that contains progressive CIN or cervical cancer (Klaes & Woerner, 1999; Cuschieri *et al.*, 2004; Molden *et al.*, 2005a; von Knebel-Doeberitz, 2002).

Cuzick showed that cytologically negative but HPV-positive women can be triaged safely by repeating cytology or HPV testing 6 to 12 months after the initial HPV positive result (Cuzick *et al.*, 2003). From the baseline results of the Finnish population trial, it was concluded that the low predictive value of a positive HC2 test for finding underlying CIN2+ could be raised to the level of cytology by cytological triage of HPV-positive women (Kotaniemi-Talonen *et al.*, 2005).

3.8.3.4 Follow-up and longitudinal performance

In screening programmes in general, the *longitudinal* performance indicators are the most relevant ones, as they determine the duration of the protective effect and thus the length of the screening interval and the cost-efficiency of the entire program.

In the Portland study, the respective longitudinal sensitivity to predict subsequent CIN3+ within 5 year or 10 years was 49% and 35% for cytology screening, 75% and 64% for HC2-based screening and 86% and 72% for combined cytological and virological testing. The 5-year cumulative risk of CIN3, was 4.4% for women who were HC2-positive at base-line, but only 0.24% among women with a negative HC2-test and 0.16% when both the HC2-test and Pap smear were negative (Sherman *et al.*, 2003). The longitudinal negative predictive value of a combined negative test, computed over a 5-year period, was very high: 99.91% (95% CI: 99.85-99.95%). This means that only 9 out of 10,000 screened subjects (95% CI: 5-15/10,000), will develop CIN3+ over a 5 year period when cytology and HPV both are negative. In women having only a negative Pap smear, this risk is 30/10,000 screened women (95% CI: 23-38/10,000).

However, the fact that the HPV test has a substantial longitudinal predictive value for future development of high-grade CIN also presents problems. To label a previously healthy woman as a high-risk individual for cancer development, requiring repeated tests at frequent intervals, is a considerable psychological stress.

It is currently unknown whether it is possible to augment clearance of HPV infection (in the absence of cytological abnormalities) by some form of treatment.

Recommendations

Further research is needed to better define the **longitudinal** performance indicators (sensitivity, specificity and positive and negative predictive values) of HPV DNA testing as well as of combined HPV DNA testing and cytology.

HPV testing in primary screening has the potential to improve effectiveness while substantially reducing the requisite number of screening episodes offered to women in a lifetime. Due to lower specificity compared to cytology-based screening, annual HPV testing must be avoided.

Research into optimal follow-up algorithms of HPV-positive women is necessary. Adequate triage methods are needed to identify those HPV-positive women that are at risk of developing cancer.

Piloting with validated HPV DNA testing can be recommended if performed in an organised screening programme with careful monitoring of the quality and systematic evaluation of the aimed outcomes, adverse effects and costs. Rollout towards national implementation can be considered only after the pilot project has demonstrated successful results with respect to effectiveness (relative sensitivity, positive predictive value of the screening test, triage and diagnostic assessment), cost-effectiveness and after key organisational problems have been resolved adequately.

3.8.3.5 What types of studies with what types of endpoints are needed?

Cross-sectional studies

This type of study is useful to establish the cross-sectional accuracy for identifying underlying high-grade CIN. Evidence, discussed in Chapter 2, indicates that cervical cancer incidence can be reduced substantially by cytological screening which consists in finding and treating high-grade CIN. Given the higher cross-sectional sensitivity (see Section 0) of HPV testing in detecting CIN2+, it can be assumed that HPV-based screening will be more effective and can result in a lower incidence of cervical cancer compared to cytological screening. However this hypothesis needs confirmation

from well conducted longitudinal studies since it is possible that a great proportion of lesions detected additionally by HPV testing are non-progressive. In that case, HPV testing would increase the risk of over-diagnosis and over-treatment.

Randomised controlled trials

Although the epidemiological evidence indicates a very substantial beneficial effect of cytological screening on cervical cancer incidence and mortality (Ponten *et al.*, 1995; IARC, 2005), the lack of prior evaluation in randomised trials has delayed the rational implementation of cervical screening programmes and has resulted in long-standing debates on the true efficacy of screening (Raffle *et al.*, 1995). To avoid this mistake in the future, new primary screening programmes should not be introduced without first performing randomised trials to investigate the effect at the population level.

In order to have direct evidence on effectiveness or at least evidence on over-diagnosis of regressive lesions, different groups of women need to be screened, managed, and treated according to different strategies, and followed over time in order to observe the eventual occurrence of disease. Randomisation is the optimal design for this purpose (IARC 2005). Mortality and incidence are the most obvious endpoints to directly assess effectiveness. However, they require large study size and duration (Davies *et al.*, 2006). This implies high costs and provides results only after many years. The health benefits may be delayed and the evaluated screening test may not even be available any more when the final study results are obtained. An alternative approach would be to take the longitudinal occurrence of high-grade CIN (particularly of CIN3) as the endpoint. This is the endpoint actually adopted in the currently running European trials. Which permits estimation of over-diagnosis with reasonable study size and duration. The effects of randomised trials may not be generalisable, when the high quality setting of trials run by dedicated scientists is different from the setting that will be used in a public health care policy (Hakama *et al.*, 1991). This can be avoided by applying the new screening strategies within the routine screening activity. An acceptable methodological approach is the randomised health care policy, which means that the new policy is not introduced for the entire target population, but only for some regions or some birth cohorts. With this strategy, research funds are not required and results apply to a real health care policy, not merely to the research setting. This approach has been successfully applied in Finland to evaluate the mammography screening program and the new cervical cancer screening tests (Hakama *et al.*, 1991; Nieminen *et al.*, 2004; Anttila *et al.*, 2006) (see also Chapter 2).

Mathematical modelling has been proposed as an alternative or a complementary tool that will provide results in a timely fashion (Royston, 1999). Although modelling studies have provided valuable information on the potential benefits of HPV screening, it is disturbing to note that different modelling studies produce substantially different results (Sherlaw-Johnson *et al.*, 1999; Cuzick *et al.*, 1999b; Myers *et al.*, 2000; Goldie *et al.*, 2004). Some of the discrepancies result from different estimates of input variables, particularly regarding cost, progression and regression rates, and sensitivity and specificity of tests. Input variables are usually estimated from the scientific literature, in which the quality and the setting of studies vary enormously. Randomised trials can be valuable for providing reliable estimates in mathematical models.

Recommendations

Advances in cervical cancer screening may be expected from judicious use of a combination of randomised trials, randomised health care policies and modelling studies. Modelling should be used to develop optimal settings and study designs for investigation of new screening strategies using intermediate endpoints (such as protection against CIN3). Effects on intermediate endpoints can then be used in further modelling studies to estimate effects on late endpoints such as mortality and to design randomised health care policies.

3.8.3.6 Ongoing European randomised trials

In population-based randomised clinical trials in five EU member states (Finland, Italy, Sweden, The Netherlands and the United Kingdom), cytology screening is currently being compared with HPV screening or combined cytology/HPV screening. In all arms of these trials the cumulative incidence of CIN2 and CIN3 in screen negatives can be assessed three to five years after initial screening. The rationale and design of the trials was recently summarised and discussed by Davies (Davies *et al.*, 2006). Some baseline results were published recently (Bulkman *et al.*, 2004; Kotaniemi-Talonen *et al.*, 2005; Elfgrén *et al.*, 2005; Anttila *et al.*, 2006; Kitchener *et al.*, 2006; Ronco *et al.*, 2006 a & b). Publication of the second round results is expected in 2007-2008. The Finnish trial will be followed-up until 2015 enabling the evaluation of incidence of invasive cervical cancer as an outcome (Anttila *et al.*, 2006).

In the Swedish trial, initially cytologically negative women 32-38 years of age with type-specific HPV persistence showed a high risk (28/100) of developing subsequent CIN2+ over an average time of 19 months. In the representative sub-cohort of women in the control arm with masked HPV status, only 2% (2/95) had CIN2/3.

In the Finnish randomised screening policy, women aged 30-60 years were screened with conventional cytology or Hybrid Capture 2 followed by cytology triage. Women were referred for colposcopy in the cytology arm if they were LSIL+, and in the HPV arm if they were HC2+ and LSIL+. The relative sensitivity (Hybrid Capture 2/cytology at LSIL+) to find CIN2+ and CIN3+ lesions was 2.0 (95% CI: 0.7-5.8) and 1.0 (95% CI: 0.3-3.1), respectively. The PPV to find CIN2+ was 4% for a positive HC2 test; 25% for a positive HC2 followed by cytology triage showing LSIL+, and 26% for the finding LSIL+ in the conventional cytology arm.

Baseline results were recently published from the Italian trial enrolling women aged 35-60 years, who were tested by conventional cytology in the conventional arm (referred to colposcopy if ASCUS+) and by Hybrid Capture 2 and LBC in the experimental arm (referred for colposcopy if either ASCUS+ or HPV+) (Ronco *et al.*, 2006b). HPV testing was more sensitive than conventional cytology (relative sensitivity for CIN2+ = 1.47; 95% CI: 1.03-2.09) but PPV was reduced (relative PPV 0.40; 95% CI: 0.23-0.60). LBC showed similar sensitivity but lower PPV (ratio = 0.57; 95% CI: 0.39-0.82) compared to conventional cytology.

Among women aged 25 to 35, HPV testing positive for HC2 at 2pg/ml cut-off with triaging by cytology allowed a significant increase in sensitivity vs. conventional cytology alone (relative sensitivity = 1.58, 95% CI: 1.03-2.44) with only small loss in PPV (relative PPV = 0.84, 95% CI: 0.56-1.25) (Ronco *et al.*, 2006a).

The outcomes of the European trials, observed in the second screening round, supplemented by mathematical modelling, and taking into account costs, psycho-social aspects and women's preferences, will be pivotal for defining future screening policy in the EU.

3.8.3.7 Using cost-effectiveness modelling to design HPV screening programmes

In a pioneering series of studies by van Ballegooijen, mathematical modelling has been used to assess which programme designs are likely to be most cost-effective, and to identify critical areas of uncertainty in which research is particularly needed (van Ballegooijen *et al.*, 1997; van Ballegooijen *et al.*, 2000; van den Akker van Marle *et al.*, 2003). Major conclusions from these studies were that the longitudinal performance of the HPV test was critical for achieving cost-efficacy, because the high cost and lower specificity of HPV screening compared to cytology-based screening needs to be compensated for by a longer screening interval in order to be cost-effective.

As discussed above, the observed lower cumulative incidence of CIN3 in women with negative baseline HPV results compared to cytologically negative women, suggests that HPV screening could be more effective. Mathematical models simulating the natural history of cervical cancer may be used to extrapolate this observed early surrogate outcome to the desired outcome (reduction of incidence and mortality from cervical cancer). The validity of such model-based simulation should be confirmed a posteriori from observed data, for example, by continued surveillance using linkage of screening histories with cancer registry data.

Mathematical models can also be used to explore the impact of multiple variables such as changes in target population, screening frequency, compliance of the population, and management options which cannot all be included in randomised trials.

Cost-effectiveness models are very instructive for decision making, but they must be based on reliable data and current local costs.

Recommendations

Design of HPV screening trials, screening policies and/or policy evaluation studies should be based on cost-effectiveness modelling studies, specific to each population to be targeted for screening.

Cost-effectiveness modelling studies should be repeated in various populations which may differ in associated costs, rates of HPV infection of different types, and background rates of other risk factors for cervical cancer.

3.8.4 Use of HPV testing in triaging women with equivocal smears

A comprehensive meta-analysis conducted in the framework of the European Network for Cervical Cancer Screening compared two triage options for women with ASCUS: repetition of the Pap smear versus immediate reflex HPV DNA testing (Arbyn *et al.*, 2002; Arbyn *et al.*, 2004a; Arbyn *et al.*, 2004b). Studies were included if data on verification by colposcopy, and biopsy in the case of colposcopic suspicion, was available for all subjects. Data from two of the three trial arms of the ASCUS-LSIL triage study (ALTS): women referred to colposcopy, and women triaged by Hybrid Capture 2, were also included (see below) (Solomon *et al.*, 2001). The ALTS was a randomised controlled trial with 3 arms, involving more than 3,000 women with an ASCUS index smear, in which three management options were compared: (1) direct referral to colposcopy, (2) HPV DNA triage using HC2, referring women being HPV positive and also those with HSIL for colposcopy and (3) repetition of the smear, referring women when the repeat smear showed HSIL or a more serious lesion (Schiffman & Adriansa, 2000). Two outcomes were considered in the meta-analysis: presence of histologically confirmed CIN2+ and CIN3+. Substantial inter-study heterogeneity was observed among studies with all systems of HPV DNA testing. By restricting the meta-analysis to studies where the Hybrid Capture 2 assay was used, the inter-study variation was reduced substantially. Recently, this meta-analysis was further updated, appending studies published until the first trimester of 2005 (Arbyn *et al.*, 2005). Some of the results of this updated pooled analysis are presented below.

The sensitivity and specificity of HC2, pooled from 16 studies, was 94% (95% CI: 92-96%) and 62% (95% CI: 56-68%), respectively. The sensitivity of HC2 was overall 14% (95% CI: 8-20%) higher than repeat cytology in six studies in which both triage methods were used (see Fig. 1). The inter-study variation of the relative sensitivity was not significant. The pooled specificity of the HC2 assay and repeat cytology were nearly equal (specificity ratio = 0.99; 95% CI: 0.89-1.11).

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Relative specificity, however, was heterogeneous among studies and varied between 0.76 and 1.18. The pooled prevalence of CIN2+ among ASCUS women was 10% (95% CI: 8-12%). Higher sensitivity of HC2 may also be concluded when CIN3+ is taken as outcome. The sensitivity and specificity for CIN3+ was 96% (95% CI: 94-98) and 56% (95% CI: 49-64%), respectively.

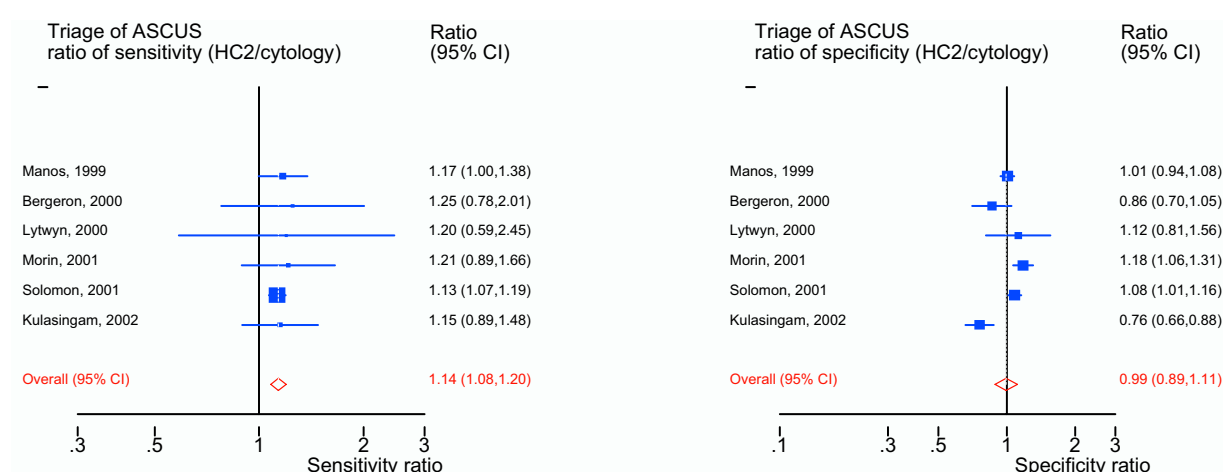


Fig. 1. Ratio of the sensitivity (at left) of triage of ASCUS cases using the HC2 assay over the sensitivity of repeat cytology, considering ASCUS (atypical squamous cells of undetermined significance) as positivity criterion, to detect histologically confirmed CIN2 or worse disease. At right: ratio of specificity¹.

To conclude, ASCUS triage using HC2 is significantly more sensitive and equally specific compared to the repetition of the Pap smear with respect to finding underlying high-grade cervical intra-epithelial neoplasia. HPV triage can be done from residual fluid used for LBC, avoiding the necessity to recall the woman. When conventional cytology is used, an additional sample for viral testing can also be taken routinely, which should only be used when the smear shows ASCUS. The cost-effect ratio (viral/cytological triage) becomes less obvious, when such an additional sample is not taken and women with ASCUS need to come for a second visit for the HPV triage test (Kim *et al.*, 2002).

This meta-analysis essentially addresses the cross-sectional accuracy for prevalent presence of high-grade CIN. The cross-sectional accuracy results of this meta-analysis are in line with those of the ALTS study, which was the largest contributing study. Nevertheless, the conclusions of the meta-analysis are robust since they do not change when the ALTS is omitted (Arbyn *et al.*, 2004a).

The ALTS also provided longitudinal follow-up data by following women with an original report of ASCUS every 6 months over a period of 2 years with serial cytology (ASCUS-LSIL Triage Study Group, 2003b). At the end, all women were submitted to colposcopy and biopsies were taken when CIN was suspected colposcopically. The 2-year cumulative diagnosis of CIN3+ was 8% to 9% in all 3 study arms. After controlling for insensitivity of colposcopy, HPV testing at enrolment showed a sensitivity of 92% (95% CI: 89-95%) for present or developing CIN3+, and 53% (95% CI: 51-55%) of women required referral for colposcopy (ASCUS-LSIL Triage Study Group, 2003b). Three successive repeat smears considering HSIL as positivity criterion, showed a sensitivity of only 60% (95% CI: 51-70%, with referral of 12% (95% CI: 10-14%) to colposcopy. When ASCUS+ was the

¹ Adapted from Arbyn M., Paraskevaides E., Martin-Hirsch P., Prendiville W., & Dillner J. (2005). Clinical utility of HPV DNA detection: triage of minor cervical lesions, follow-up of women treated for high-grade CIN. An update of pooled evidence. *Gynecol.Oncol.* **99** (Suppl 3): 7-11 with permission from Elsevier.

cutoff, the sensitivity of repeat cytology was 97% (95% CI: 94-100%), with referral of 73% (95% CI: 70-75%) to colposcopy.

In conclusion, serial cytology every six months, with a cut-off of ASCUS or worse, is as sensitive as one reflex HPV DNA test immediately after a first observation of ASCUS. However, the high sensitivity of repeat cytology is dependent on the compliance with multiple follow-up visits and involves high costs for repeat visits and referral colposcopy.

A caveat should be mentioned concerning reflex HPV triage in young women less than 25 years of age. In this age-group, prevalence and also clearance of HPV usually is high, whereas the probability of having a progressive high-grade abnormality is low (Boardman *et al.*, 2005; Sawaya, 2005; Wright *et al.*, 2006).

Recommendations

Triage with a non-specific, validated HPV test is a recommended management option for a cytological result of ASCUS.¹ Repeat cytology is still an acceptable option if compliance with follow-up recommendations can be assured, or if HPV tests are not available.

3.8.5 Use of HPV testing in triaging women with LSIL

Another meta-analysis, sponsored by the European Commission and the Cochrane Gynaecological Cancer Review Group, concerned management of women with a cytological result of LSIL.

The cross-sectional sensitivity of viral triage to detect high-grade CIN, using HC2, derived from 10 published reports, was high (97%, 95% CI: 96-99%), whereas the pooled specificity was low (29%, 95% CI: 22-36%) (Arbyn *et al.*, 2005). The overall relative sensitivity of the HPV testing compared to repeat cytology (derived from only four studies) was 1.07 (95% CI: 0.92-1.25), indicating non significantly higher sensitivity for the virological triage method (see Fig. 2). Moreover, HC2 showed a substantially and significantly lower specificity than the repeat Pap smear (pooled specificity ratio of 0.60 (95% CI: 0.36-0.99)). In the ALTS study, the specificity of HC2, at its usual cut-off of 1pg HPV DNA/ml was extremely low among women younger than 29 years (14%), but it was also low among women aged 30 or older (26%) (Sherman *et al.*, 2002). The pooled prevalence of CIN2+ among women with LSIL was 19% (95% CI: 12-25%). The HPV positivity rate was very high in most studies. Its pooled value was 77% (95% CI: 71-82%).

For this reason, the recruitment of women with LSIL for the ALTS study was prematurely interrupted (ALTS group & Anonymous, 2000). Nevertheless, follow-up of already enrolled women and women with ASCUS continued. The sensitivity of HC2 at enrolment and serial cytology at 6-month intervals over 2 years (at ASCUS+) to detect prevalent or developing CIN3+ were both very high: 95% (95% CI: 92-98%) and 100%, respectively. The rate of referral for colposcopy was 84% when HPV triage was used and 89% (95% CI: 87-91%) when repetitive cytology was offered.

Repetition of the HC2 test one year after the index LSIL report detected 92% of cumulative CIN3+ and was associated with a referral rate of 55% (Cox *et al.*, 2003; Guido *et al.*, 2003).

Further research is needed to identify sensitive markers that are also specific for the risk of having or developing high-grade CIN. A posteriori HPV typing of the ALTS samples indicated that typing for more than 10 HPV genotypes involves serious loss in specificity with virtually no additional gain in sensitivity (Schiffman *et al.*, 2005b). On the other hand, finding of just HPV16, and maybe some

¹ This recommendation is also valid for the cytological result of "ASC-US", as defined in The Bethesda 2001 terminology (Arbyn *et al.*, 2004a). For the explanation of "ASCUS" and "ASC-US" see the Glossary ("Atypical squamous cells of undetermined significance"), Annex 2 of this chapter, and Solomon *et al.*, 2002.

other highly oncogenic HPV types might indicate a serious risk of progression, especially when viral load is high and women are not too young. In the ALTS, the 2-year cumulative risk for CIN3+ was 39% (95% CI: 34-45%) among LSIL women who carried HPV 16 (Castle *et al.*, 2005b). This risk was only 10% (95% CI: 8-12%) among LSIL women being positive for HC2 but negative for HPV 16, which was even lower than the risk without knowing the HPV status.

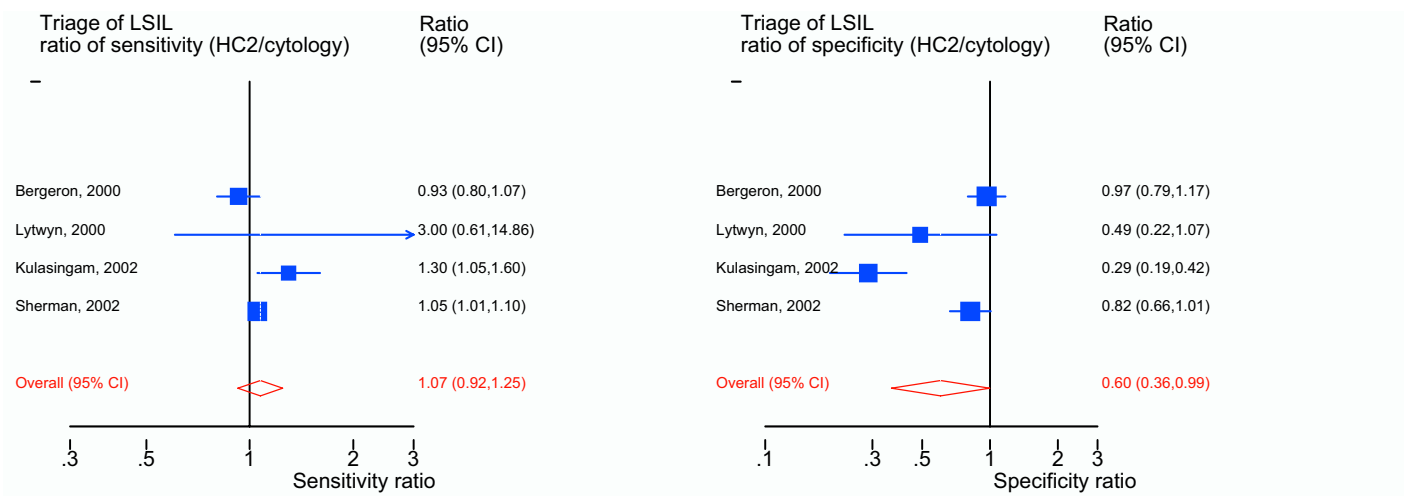


Fig. 2. Ratio of the sensitivity (at left) of triage of LSIL cases using the HC2 assay over the sensitivity of repeat cytology, considering ASCUS (atypical squamous cells of undetermined significance) as positivity criterion, to detect histologically confirmed CIN2 or worse disease. At right: ratio of specificity¹

Other molecular surrogate progression markers, cell cycle regulation proteins, viral integration markers and presence of viral RNA coding for E6 or E7 oncoproteins are interesting potential candidates for LSIL triage to be evaluated in trials.

Recommendations

Reflex HPV triage using a non-specific HPV-test is, in general, not a useful management option in case of LSIL. Nevertheless, reflex HPV testing may be cost effective in older women with LSIL, due to considerably lower prevalence of HPV infection. Repetition of cytology at 6 to 12 months or HPV testing at 12 months, with or without colposcopy, are possible management options. Research is needed to identify a good reflex triage test for women with LSIL. Future study findings should be reported with sufficient age stratification.

3.8.6 Use of HPV testing in follow-up after treatment of CIN

The accuracy of HPV DNA testing and follow-up cytology in predicting residual or recurrent disease after treatment were compared in two reviews (Paraskevaidis *et al.*, 2004; Zielinski *et al.*, 2004). These reviews were recently updated and the results combined in a formal meta-analysis (Arbyn *et al.*, 2005). Treatment procedures for CIN are discussed in Chapter 6.

¹ Adapted from Arbyn M., Paraskevaidis E., Martin-Hirsch P., Prendiville W., & Dillner J. (2005). Clinical utility of HPV DNA detection: triage of minor cervical lesions, follow-up of women treated for high-grade CIN. An update of pooled evidence. *Gynecol.Oncol.* **99 (Suppl 3)**: 7-11 with permission from Elsevier.

Excision is effective and enables histopathological examination of the resulting cone biopsy contrary to ablative procedures. Evaluation of the excision margin status does not reliably predict residual or persistent disease (Jansen *et al.*, 1994; Soutter *et al.*, 1997). Recurrent CIN is reported in 0.3-23% of women with free cone margins and in 6.9-84.8% of women without free margins (Dobbs *et al.*, 2000; Paraskevaidis *et al.*, 2000; Gonzalez *et al.*, 2001; Paraskevaidis *et al.*, 2001a). Follow-up using Pap smears after treatment is still the most common procedure but has been questioned because of relatively low and variable sensitivity for CIN detection (Martin-Hirsch *et al.*, 2002). Because HPV infection is essential for development and maintenance of CIN, HPV DNA detection may predict residual of recurrent CIN more rapidly and with higher sensitivity. Several studies have found that HPV DNA is commonly cleared after effective treatment for CIN and that persistence of HPV DNA predicts recurrence (Elfgren *et al.*, 1996; Chua & Hjerpe, 1997; Kanamori & Kigawa, 1998; Mann *et al.*, 2001). These studies have used several different treatment modalities, which were found to have varying success in clearance of HPV DNA, suggesting that HPV DNA testing is useful for evaluation of different treatment modalities.

There was marked heterogeneity of the studies included in the reviews with respect to the grade of CIN treated, treatment procedure, the method of HPV testing, the assessment of the outcome (residual or recurrent CIN) and the timing and duration of follow-up.

Nevertheless, some consistent conclusions were evident. Overall, HPV DNA testing after treatment was positive in 95% (95% CI: 91-99%; range: 70-100%) of cases with residual or recurring CIN during study follow-up. Treatment failure was predicted by HPV testing with higher sensitivity than cytology in 7 of 9 included studies (Elfgren *et al.*, 1996; Chua & Hjerpe, 1997; Nagai *et al.*, 2000; Nobbenhuis *et al.*, 2001; Jain *et al.*, 2001; Paraskevaidis *et al.*, 2001a; Bar-Am *et al.*, 2003; Zielinski *et al.*, 2003). In 4 studies, the difference was significant (Chua & Hjerpe, 1997; Nobbenhuis *et al.*, 2001; Jain *et al.*, 2001; Paraskevaidis *et al.*, 2001a). In one study, the sensitivity of HPV testing was lower but not significantly lower (Bekkers *et al.*, 2002) and in another study it was equal to cytology (Zielinski *et al.*, 2003). Overall, the sensitivity ratio (HPV/cytology) was 1.27 (95% CI: 1.06-1.27). The pooled specificity of HPV testing was not significantly lower than that of follow-up cytology (ratio: 0.94; 95% CI: 0.87-1.01).

HPV DNA testing was also significantly more sensitive (ratio: 1.30; 95% CI: 1.05-1.62) and not significantly more specific (ratio: 1.09; 95% CI: 0.98-1.22) than the histological assessment of margins of the excised tissue (Fig. 3).

In summary, there is evidence that post-treatment HPV testing can predict treatment failure with significantly higher sensitivity and similar specificity compared to repeat cytology and the histological status of the margins.

Timing of taking a post-treatment HPV test

The optimal timing for a post-treatment HPV test has been explored in only a few studies (cf., Nobbenhuis *et al.*, 2001; Elfgren *et al.*, 2002). A substantial proportion of women show clearance already at 3 months and clearance is also significant between 3 and 6 months. After 6 months, the clearance rate is lower, however. An extensive evaluation of possible post-treatment testing options in the Dutch screening program that previously used cytology at 6, 12 and 24 months post treatment found evidence to suggest that double testing with cytology and HPV at 6 months and 24 months would be more efficient (Zielinski *et al.*, 2003).

Until recently, knowledge of the long-term outcome after treatment of CIN was limited. A recent meta-analysis and a new retrospective cohort study linking cancer registry data with treatment histories showed an increased risk of invasive cervical cancer (relative risk of 2 to 3) until 10 and even 20 years after conservative cervical treatment (Soutter *et al.*, 2005; Kalliala *et al.*, 2005). These data clearly demonstrate the need to continue research for post-treatment follow-up strategies.

Recommendations

Since women who have been treated for CIN still have an increased risk for invasive cervical cancer, there is a definite need for improved follow-up regimens. The use of post-treatment HPV testing should be explored in designing new regimens for CIN treatment follow-up.

There is evidence for improvement of post treatment follow-up at six months by double testing with cytology and an HPV test. Evidence also suggests that subsequent follow-up of women negative for both HPV and cytology should be less intense, but it is not currently possible to recommend which regimen would be more effective. Implementation and careful monitoring and/or randomisation of subsequent follow-up regimens is recommended.

Further research on the long-term protection of HPV-negativity, and of joint cytology- and HPV-negativity is warranted.

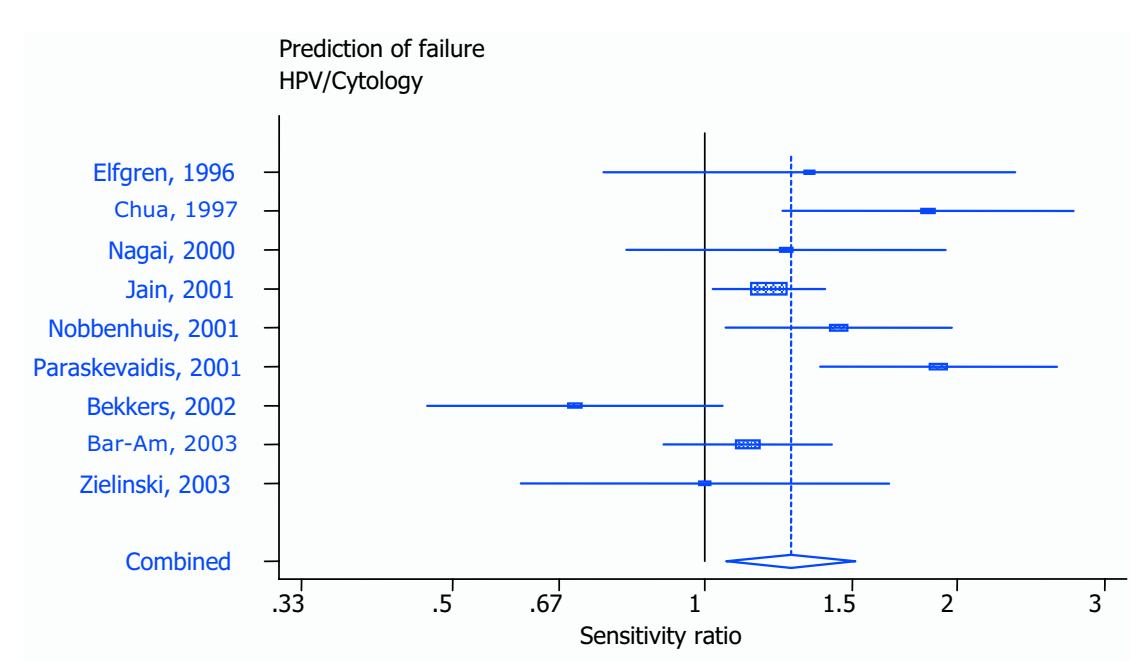


Fig. 3. Meta-analysis of the sensitivity of HPV testing relative to follow-up cytology to detect residual or recurrent disease after treatment of high-grade CIN¹.

3.9 Conclusions

The following conclusions can be drawn from the reviews outlined in this chapter and in Chapter 2.

In well organised settings, with a high level of quality assurance, conventional cytological screening reduces the incidence of squamous cervical cancer by 80% or more. (outcome 1-3; study types 2-

¹ Adapted from Arbyn M., Paraskevaidis E., Martin-Hirsch P., Prendiville W., & Dillner J. (2005). Clinical utility of HPV DNA detection: triage of minor cervical lesions, follow-up of women treated for high-grade CIN. An update of pooled evidence. *Gynecol.Oncol.* **99 (Suppl 3)**: 7-11 with permission from Elsevier.

4, see Table 1). Nevertheless, drawbacks of cytological screening are its low-to-moderate reproducibility and highly variable cross-sectional sensitivity for high-grade lesions. Therefore, the quality assurance measures recommended in this guideline need to be fully implemented in cytological screening.

The sensitivity and specificity of liquid-based cytology is similar to conventional cytology in detection of high-grade CIN. The percentage of unsatisfactory smears usually is lower and the interpretation requires less time compared with conventional smears. The quality of evaluation reported in the literature is quite poor (outcome 6, study type 2).

One large population-based randomised trial which compared automated cytology with high-quality manual conventional cytology showed equal sensitivity and specificity for high-grade CIN and cancer (outcome 5, study types 1-2).

HPV DNA testing with validated methods is highly reproducible. The high-risk cocktail of Hybrid-Capture II is more sensitive and equally specific compared to repeat cytology in triage of women with equivocal cytology to ascertain the need for further management. Most women with LSIL are HPV positive, limiting the efficiency of reflex HPV triage. After conservative treatment of cervical lesions, HPV testing picks up residual or recurrent CIN more quickly than follow-up cytology, with higher sensitivity and not lower specificity than follow-up cytology.

Primary screening with HC2 or validated PCR systems is substantially more sensitive in identifying CIN2, CIN3, or cancer than cytology at cut-off ASCUS or LSIL, but it is less specific. The specificity of HPV screening can be enhanced by targeting women older than 30-35 years. Combining HPV and cytology screening yields a small gain in sensitivity for high-grade CIN lesions, but at the expense of a considerable loss in specificity, compared to isolate HC2 screening (outcome 4-6, study types 1-3, study type 1 only for outcomes 5-6). Potential methods to triage HPV-positive women are: cytology, repetition of the HPV test 6-12 months later, typing for a limited set of HPV types (including HPV 16), assessment of the (type-specific) viral load, viral integration, mRNA or cell-cycle regulating proteins. Identification of the best triage option is still a subject of research.

Current randomised controlled trials may demonstrate lower cumulative incidence of CIN3 and invasive cervical cancer as joint or separate outcomes in HPV-negative compared to cytology-negative women. The results of these trials are needed before screening policies for primary HPV screening can be recommended in Europe. Such policies would also have to ensure that possible increases in the detection and management of less severe lesions are kept to an appropriate minimum.

Primary HPV screening should not be recommended without specifying the age group to be targeted, the screening interval, and the essential elements of quality assurance required for programme implementation. HPV screening in an opportunistic setting is not recommended, because adherence to the appropriate intervals and requisite quality control cannot be adequately assured under such conditions.

Piloting with validated HPV DNA testing can be recommended if performed in an organised screening programme with careful monitoring of the quality and systematic evaluation of the aimed outcomes, adverse effects and costs. Rollout towards national implementation can be considered only after the pilot project has demonstrated successful results with respect to effectiveness (relative sensitivity, positive predictive value of the screening test, triage and diagnostic assessment) and cost-effectiveness, and after key organisational problems have been adequately resolved.

3.10 References

Agorastos T., Dinas K., Lloveras B., de Sanjose S., Kornegay J.R., Bonti H., Bosch F.X., Constantinidis T., & Bontis J. (2005). Human papillomavirus testing for primary screening in women at low risk of developing cervical cancer. The Greek experience. *Gynecol. Oncol.* **96**: 714-720.

ALTS group & Anonymous (2000). Human papillomavirus testing for triage of women with cytologic evidence of low-grade squamous intraepithelial lesions: baseline data from a randomized trial. *J. Natl. Cancer Inst.* **92**: 397-402.

Anttila A., Hakama M., Kotaniemi-Talonen L., & Nieminen P. (2006). Alternative technologies in cervical cancer screening: a randomised evaluation trial. *BMC. Public Health* **6**: (152) 1-8.

Arbyn M. & Abarca M. (2003, updated in 2005). Is Liquid Based Cytology an Effective Alternative for the Conventional Pap Smear to Detect Cervical Cancer Precursors? A Systematic Review and Meta-analysis. IPH/EPI-REPORTS **10**, 1-201. Brussels, Scientific Institute of Public Health.

Arbyn M., Buntinx F., Van Ranst M., & Cortiñas Abrahantes J. (2002). Triage of women with atypical or low-grade cytological abnormalities of the cervix by HPV testing: systematic review and meta-analysis. IPH/EPI-REPORTS Nr. **2001-019**, 1-240. Brussels, Scientific Institute of Public Health.

Arbyn M., Buntinx F., Van Ranst M., Paraskevaidis E., Martin-Hirsch P., & Dillner J. (2004a). Virologic versus cytologic triage of women with equivocal Pap smears: a meta-analysis of the accuracy to detect high-grade intraepithelial neoplasia. *J. Natl. Cancer Inst.* **96**: 280-293.

Arbyn M., Dillner J., Van Ranst M., Buntinx F., Martin-Hirsch P., & Paraskevaidis E. (2004b). Re: Have we resolved how to triage equivocal cervical cytology? *J. Natl. Cancer Inst.* **96**: 1401-1402.

Arbyn M., Paraskevaidis E., Martin-Hirsch P., Prendiville W., & Dillner J. (2005). Clinical utility of HPV DNA detection: triage of minor cervical lesions, follow-up of women treated for high-grade CIN. An update of pooled evidence. *Gynecol. Oncol.* **99 (Suppl 3)**: 7-11.

Arbyn M., Sasieni P., Meijer C.J., Clavel C., Koliopoulos G., & Dillner J. (2006). Chapter 9: Clinical applications of HPV testing: A summary of meta-analyses. *Vaccine* **24 S3**: 78-89.

ASCUS-LSIL Triage Study Group (2003a). A randomized trial on the management of low-grade squamous intraepithelial lesion cytology interpretations. *Am. J. Obstet. Gynecol.* **188**: 1393-1400.

ASCUS-LSIL Triage Study Group (2003b). Results of a randomized trial on the management of cytology interpretations of atypical squamous cells of undetermined significance. *Am. J. Obstet. Gynecol.* **188**: 1383-1392.

Austin R.M. & Ramzy I. (1998). Increased detection of epithelial cell abnormalities by liquid-based gynecologic cytology preparations. *Acta Cytol.* **42**: 178-184.

Bar-Am A., Gamzu R., Levin I., Fainaru O., Niv J., & Almog B. (2003). Follow-up by combined cytology and human papillomavirus testing for patients post-cone biopsy: results of a long-term follow-up. *Gynecol. Oncol.* **91**: 149-153.

Begg C.B. & Greenes R.A. (1983). Assessment of diagnostic tests when disease verification is subject to selection bias. *Biometrics* **39**: 207-215.

- Bekkers R.L., Melchers W.J., Bakkers J.M., Hanselaar A.G., Quint W.G., Boonstra H., & Massuger L.F. (2002). The role of genotype-specific human papillomavirus detection in diagnosing residual cervical intraepithelial neoplasia. *Int. J. Cancer* **102**: 148-151.
- Bergeron C., Bishop J., Lemarie A., Cas F., Ayivi J., Huynh B., & Barrasso R. (2001). Accuracy of Thin-Layer Cytology in Patients Undergoing Cervical Cone Biopsy. *Acta Cytol.* **45**: 519-524.
- Bergeron C., Masseroli M., Ghezi A., Lemarie A., Mango L., & Koss L.G. (2000). Quality control of cervical cytology in high-risk women. PAPNET system compared with manual rescreening. *Acta Cytol.* **44**: 151-157.
- Bigras G. & De Marval F. (2005). The probability for a Pap test to be abnormal is directly proportional to HPV viral load: results from a Swiss study comparing HPV testing and liquid-based cytology to detect cervical cancer precursors in 13 842 women. *Br. J. Cancer* **93**: 575-581.
- Bigras G., Rieder M.A., Lambercy J.-M., Kunz B., Chatelain J.-P., Reymond O., & Cornaz D. (2003). Keeping collecting device in liquid medium is mandatory to ensure optimized liquid-based cervical cytologic sampling. *J. Low Genit. Tract. Dis.* **7**: 168-174.
- Blumenthal P.D., Gaffikin L., Chirenje Z.M., McGrath J., Womack S., & Shah K. (2001). Adjunctive testing for cervical cancer in low resource settings with visual inspection, HPV, and the Pap smear. *Int. J. Gynecol. Obstet.* **72**: 47-53.
- Boardman L.A., Stanko C., Weitzen S., & Sung J. (2005). Atypical squamous cells of undetermined significance: human papillomavirus testing in adolescents. *Obstet. Gynecol.* **105**: 741-746.
- Boon M.E. & Suurmeijer A.J.H. (1993). The Pap Smear, second edition. Coulomb Press Leyden.
- Bos A.B., van Ballegooijen M., van den Akker van Marle M.E., Hanselaar A.G., van Oortmarssen G.J., & Habbema J.D. (2001). Endocervical status is not predictive of the incidence of cervical cancer in the years after negative smears. *Am. J. Clin. Pathol.* **115**: 851-855.
- Bosch F.X., Lorincz A.T., Muñoz N., Meijer C.J., & Shah K.V. (2002). The causal relation between human papillomavirus and cervical cancer. *J. Clin. Pathol.* **55**: 244-265.
- Bossuyt P.M., Reitsma J.B., Bruns D.E., Gatsonis C.A., Glasziou P.P., Irwig L.M., Lijmer J.G., Moher D., Rennie D., & de Vet H.C. (2003). Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. *BMJ* **326**: 41-44.
- Boyes D.A., Morrison B., Knox E.G., Draper G.J., & Miller A.B. (1982). A cohort study of cervical cancer screening in British Columbia. *Clin. Invest. Med.* **5**: 1-29.
- BSCC Editorial (1990). Cell content of cervical smears. *Cytopathology* **1**: 129-130.
- Bulkmans N.W., Rozendaal L., Snijders P.J., Voorhorst F.J., Boeke A.J., Zandwijken G.R., van Kemenade F.J., Verheijen R.H., Groningen K., Boon M.E., Keuning H.J., van Ballegooijen M., van den Brule A.J., & Meijer C.J. (2004). POBASCAM, a population-based randomized controlled trial for implementation of high-risk HPV testing in cervical screening: design, methods and baseline data of 44,102 women. *Int. J. Cancer* **110**: 94-101.
- Carcheri M., Lacitgnola G., Riva R., Capuzzo R., Mentasti M., Ventura A., & et al (2003). HPV-DNA research and viral genotyping in diagnosis and prevention of uterine cervix carcinoma. *Microbiol. Med.* **18**: 39-42.
- Carozzi F.M., Del Mistro A., Confortini M., Sani C., Puliti D., Trevisan R., De Marco L., Tos A.G.,

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Girlando S., Palma P.D., Pellegrini A., Schiboni M.L., Crucitti P., Pierotti P., Vignato A., & Ronco G. (2005). Reproducibility of HPV DNA Testing by Hybrid Capture 2 in a Screening Setting. *Am. J. Clin. Pathol.* **124**: 716-721.

Castle P.E., Lorincz A.T., Mielzynska-Lohnas I., Scott D.R., Glass A.G., Sherman M.E., Schussler J.E., & Schiffman M.A. (2002a). Results of human papillomavirus DNA testing with the hybrid capture 2 assay are reproducible. *J. Clin. Microbiol.* **40**: 1088-1090.

Castle P.E., Schiffman M.A., Burk R.D., Wacholder S., Hildesheim A., Herrero R., Bratti M.C., Sherman M.E., & Lorincz A. (2002b). Restricted cross-reactivity of hybrid capture 2 with non-oncogenic human papillomavirus types. *Cancer Epidemiol. Biomarkers Prev.* **11**: 1394-1399.

Castle P.E., Schiffman M.A., Herrero R., Hildesheim A., Rodriguez A.C., Bratti M.C., Sherman M.E., Wacholder S., Tarone R., & Burk R.D. (2005a). A prospective study of age trends in cervical human papillomavirus acquisition and persistence in Guanacaste, Costa Rica. *J. Infect. Dis.* **191**: 1808-1816.

Castle P.E., Solomon D., Schiffman M., & Wheeler C.M. (2005b). Human papillomavirus type 16 infections and 2-year absolute risk of cervical precancer in women with equivocal or mild cytologic abnormalities. *J. Natl. Cancer Inst.* **97**: 1066-1071.

Chock C., Irwig I., Berry G., & Glasziou P. (1997). Comparing dichotomous screening tests when individuals negative on both tests are not verified. *J. Clin. Epidemiol.* **50**: 1211-1217.

Choi B.C. (1992). Sensitivity and specificity of a single diagnostic test in the presence of work-up bias. *J. Clin. Epidemiol.* **45**: 581-586.

Chua K., Wiklund F., Lenner P., Angstrom T., Hallmans G., Bergman F., Sapp M., Schiller J., Wadell G., Hjerpe A., & Dillner J. (1996). A prospective cohort study on the risk to develop cervical intraepithelial neoplasia among healthy subjects with serum antibodies to HPV, in comparison with presence of HPV DNA in cervical smears. *Int. J. Cancer* **68**: 54-59.

Chua K.L. & Hjerpe A. (1996). Persistence of human papillomavirus (HPV) infections preceding cervical carcinoma. *Cancer* **77**: 121-127.

Chua K.L. & Hjerpe A. (1997). Human papillomavirus analysis as a prognostic marker following conization of the cervix uteri. *Gynecol. Oncol.* **66**: 108-113.

Clavel C., Masure M., Bory J.-P., Putaud I., Mangeonjean C., Lorenzato M., Gabriel R., Quereux C., & Birembaut P. (1999). Hybrid capture II-based human papillomavirus detection, a sensitive test to detect in routine highgrade cervical lesions : a preliminary study on 1518 women. *Br. J. Cancer* **80**: 1306-1311.

Clavel C., Masure M., Bory J.-P., Putaud I., Mangeonjean C., Lorenzato M., Nazeyrollas P., Gabriel R., Quereux C., & Birembaut P. (2001). Human papillomavirus testing in primary screening for the detection of high-grade cervical lesions: a study of 7932 women. *Br. J. Cancer* **89**: 1616-1623.

Cochrane Methods Group on Systematic Review of Screening and Diagnostic Tests (1996). The Cochrane methods working group on systematic review of screening and diagnostic tests: recommended methods. 1-15.

Confortini M., Bulgaresi P., Cariaggi M.P., Carozzi F.M., Cecchini S., Cipparrone I., Iossa A., Maddau C., Mancini M., Sani C., Troni M., Zappa M., & Ciatto S. (2004). Comparing conventional and liquid-based smears from a consecutive series of 297 subjects referred to colposcopy assessment. *Cytopathology* **15**: 168-169.

- Coste J., Cochand-Priollet B., de Cremoux P., Le Gales C., Cartier I., Molinie V., Labbe S., Vacher-Lavenu M.C., & Vielh P. (2003). Cross sectional study of conventional cervical smear, monolayer cytology, and human papillomavirus DNA testing for cervical cancer screening. *BMJ* **326**: 733-736.
- Cox J.T., Schiffman M.A., & Solomon D. (2003). Prospective follow-up suggests similar risk of subsequent cervical intraepithelial neoplasia grade 2 or 3 among women with cervical intraepithelial neoplasia grade 1 or negative colposcopy and directed biopsy. *Am. J. Obstet. Gynecol.* **188**: 1406-1412.
- Cuschieri K.S., Whitley M.J., & Cubie H.A. (2004). Human papillomavirus type specific DNA and RNA persistence--implications for cervical disease progression and monitoring. *J. Med. Virol.* **73**: 65-70.
- Cuzick J., Beverley E., Ho L., Terry G., Sapper H., Mielzynska I., Lorincz A.T., Chan W.K., Krausz T., & Soutter P. (1999a). HPV testing in primary screening of older women. *Brit. J. Cancer* **81**: 554-558.
- Cuzick J., Clavel C., Petry K.U., Meijer C.J., Hoyer H., Ratnam S., Szarewski A., Birembaut P., Kulasingam S., Sasieni P., & Iftner T. (2006). Overview of the European and North American studies on HPV testing in primary cervical cancer screening. *Int. J. Cancer* **119**: 1095-1101.
- Cuzick J., Sasieni P., Davies P., Adams J., Normand C., Frater A., van Ballegooijen M., & van den Acker E. (1999b). A systematic review of the role of human papillomavirus testing within a cervical screening programme. *Health Technol. Assess.* **3**: 1-204.
- Cuzick J., Szarewski A., Cubie H., Hulman G., Kitchener H., Luesley D., McGoogan E., Menon U., Terry G., Edwards R., Brooks C., Desai M., Gie C., Ho L., Jacobs I., Pickles C., & Sasieni P. (2003). Management of women who test positive for high-risk types of human papillomavirus: the HART study. *Lancet* **362**: 1871-1876.
- Cuzick J., Szarewski A., Terry G., Ho L., Hanby A., Maddox P., Anderson M., Kocjan G., Steele S.T., & Guillebaud J. (1995). Human papillomavirus testing in primary cervical screening. *Lancet* **345**: 1533-1536.
- Davey D.D., Austin R.M., Birdsong G., Buck H.W., Cox J.T., Darragh T.M., Elgert P.A., Hanson V., Henry M.R., & Waldman J. (2002). ASCCP patient management guidelines: Pap test specimen adequacy and quality indicators. *Am. J. Clin. Pathol.* **118**: 714-718.
- Davies P., Arbyn M., Dillner J., Kitchener H., Ronco G., & Hakama M. (2006). A report on the current status of European research on the use of human papillomavirus testing for primary cervical cancer screening. *Int. J. Cancer* **118**: 791-796.
- Day N., Moss S., Berrino F., Choi N.W., Clarke E.A., Döbrösy L., Geirsson G., Habbema D.F., Hakama M., Hougen A., Johannesson G., Langmark F., Macgregor J.E., Magnus K., Malke B., Jensen O.M., Nelson N.A., Parkin D.M., Pettersson F., Poll P., Prorok P.C., Raymond L., & van Oortmarssen G.J. (1986). Screening for squamous cervical cancer: duration of low risk after negative results of cervical cytology and its implication for screening policies. *BMJ* **293**: 659-664.
- Day N.E. (1989). Screening for cancer of the cervix. *J. Epidemiol. Community Health* **43**: 103-106.
- de Sanjose S., Almirall R., Lloveras B., Font R., Diaz M., Muñoz N., Catala I., I, Meijer C.J., Snijders P.J., Herrero R., & Bosch F.X. (2003). Cervical Human Papillomavirus Infection in the Female Population in Barcelona, Spain. *Sex. Transm. Dis.* **30**: 788-793.
- DeMay R.M. (1996). Cytopathology of false negatives preceding cervical carcinoma. *Am. J. Obstet. Gynecol.* **175**: 1110-1113.

Dobbs S.P., Asmussen T., Nunns D., Hollingworth D., Brown L.J., & Ireland D. (2000). Does histological incomplete excision of cervical intraepithelial neoplasia following large loop excision of transformation zone increase recurrence rates? A six year cytological follow up. *BJOG* **107**: 1298-1301.

Doornewaard H., van der Schouw Y.T., van der Graaf Y., Bos A.B., Habbema J.D.F., & van den Tweel J.G. (1999). The Diagnostic Value of Computer-Assisted Primary Cervical Smear Screening: A Longitudinal Cohort Study. *Mod. Pathol.* **12**: 995-1000.

Duggan M.A. (2000). Papnet-assisted, primary screening of cervico-vaginal smears. *Eur. J. Gynaecol. Oncol.* **21**: 35-42.

Dunton C.J. (2000). New technology in Papanicolaou smear processing. *Clin Obstet Gynecol* **43**: 410-417.

Elfgren K. Longitudinal studies of human papillomavirus infection (2003). Doctoral thesis. Karolinska Institutet, Stockholm, Sweden

Elfgren K., Bistoletti P., Dillner L., Walboomers J.M., Meijer C.J., & Dillner J. (1996). Conization for cervical intraepithelial neoplasia is followed by disappearance of human papillomavirus deoxyribonucleic acid and a decline in serum and cervical mucus antibodies against human papillomavirus antigens. *Am. J. Obstet. Gynecol.* **174**: 937-942.

Elfgren K., Jacobs M., Walboomers J.M., Meijer C.J., & Dillner J. (2002). Rate of human papillomavirus clearance after treatment of cervical intraepithelial neoplasia. *Obstet. Gynecol.* **100**: 965-971.

Elfgren K., Rylander E., Radberg T., Strander B., Strand A., Paaanen K., Sjoberg I., Ryd W., Silins I., & Dillner J. (2005). Colposcopic and histopathologic evaluation of women participating in population-based screening for human papillomavirus deoxyribonucleic acid persistence. *Am. J. Obstet. Gynecol.* **193**: 650-657.

Elias A., Linthorst G., Bekker B., & Vooijs P.G. (1983). The significance of endocervical cells in the diagnosis of cervical epithelial changes. *Acta Cytol.* **27**: 225-229.

Etherington I.J., Luesley D.M., Shafi M.I., Dunn J., Hiller L., & Jordan J.A. (1997). Observer variability among colposcopists from the West Midlands region. *Br. J. Obstet. Gynaecol.* **104**: 1380-4.

Evander M., Edlund K., Gustafsson A., Jonsson M., Karlsson R., Rylander E., & Wadell G. (1995). Human papillomavirus infection is transient in young women: a population-based cohort study. *J. Infect. Dis.* **171**: 1026-1030.

Fahey M.T., Irwig L., & Macaskill P. (1995). Meta-analysis of Pap test accuracy. *Am. J. Epidemiol.* **141**: 680-689.

Ferenczy A. & Franco E.L. (1997). Human Papillomavirus DNA testing using liquid-based cytology. In: *New Developments in Cervical Cancer Screening and Prevention* (eds Franco E.L. & Monsonego J.), pp. 343-353. Blackwell Science, Cambridge.

Ferenczy A., Franco E.L., Arseneau J., Wright T.C., & Richart R.M. (1996a). Diagnostic performance of Hybrid Capture human papillomavirus deoxyribonucleic acid assay combined with liquid-based cytologic study. *Am. J. Obstet. Gynecol.* **175**: 651-656.

Ferenczy A., Robitaille J., Franco E.L., Arseneau J., Richart R.M., & Wright T.C. (1996b).

Conventional cervical cytologic smears vs. ThinPrep smears. A paired comparison study on cervical cytology. *Acta Cytol.* **40**: 1136-1142.

Forslund O. & Antonsson A. Population-based type-specific prevalence of high risk human papillomavirus infection in middle-aged Swedish women. 18th International Papillomavirus Conference Abstract Book , 241. 2000. Barcelona

Gage J.C., Hanson V.W., Abbey K., Dippery S., Gardner S., Kubota J., Schiffman M., Solomon D., & Jeronimo J. (2006). Number of cervical biopsies and sensitivity of colposcopy. *Obstet. Gynecol.* **108**: 264-272.

Geyer JW, Carrico C, Bishop JW (2000). Cellular constitution of Autocyte PREP[®] cervico-vaginal samples with biopsy-confirmed HSIL. *Acta Cytol.*; **44**:505 (abstract).

Gillio-Tos A., De Marco L., Ghisetti V., Snijders P.J., Segnan N., Ronco G., & Merletti F. (2006). Human papillomavirus typing with GP5+/6+ polymerase chain reaction reverse line blotting and with commercial type-specific PCR kits. *J. Clin. Virol.* **36**:126-132

Goldie S.J., Kim J.J., & Wright T.C. (2004). Cost-effectiveness of human papillomavirus DNA testing for cervical cancer screening in women aged 30 years or more. *Obstet. Gynecol.* **103**: 619-631.

Gonzalez D.I.Jr., Zahn C.M., Retzlöff M.G., Moore W.F., Kost E.R., & Snyder R.R. (2001). Recurrence of dysplasia after loop electrosurgical excision procedures with long-term follow-up. *Am. J. Obstet. Gynecol.* **184**: 315-321.

Gravitt P.E., Peyton C.L., Apple R.J., & Wheeler C.M. (1998). Genotyping of 27 human papillomavirus types by using L1 consensus PCR products by a single-hybridization, reverse line blot detection method. *J. Clin. Microbiol.* **36**: 3020-3027.

Guido R., Schiffman M.A., Solomon D., & Burke L. (2003). Postcolposcopy management strategies for women referred with low-grade squamous intraepithelial lesions or human papillomavirus DNA-positive atypical squamous cells of undetermined significance: a two-year prospective study. *Am. J. Obstet. Gynecol.* **188**: 1401-1405.

Hagmar B., Kalantari M., Skyldberg B., Moberger B., Johansson B., Walaas L., & Warleby B. (1995). Human papillomavirus in cell samples from the Stockholm gynecological health screening. *Acta Cytol.* **39**: 741-745.

Hakama M., Chamberlain J., Day N.E., Miller A.B., & Prorok P.C. (1985). Evaluation of screening programmes for gynaecological cancer. *Br. J. Cancer* **52**: 669-673.

Hakama M., Elovainio L., Kajantie R., & Louhivuori K. (1991). Breast cancer screening as public health policy in Finland. *Br. J. Cancer* **64**: 962-964.

Hakama M., Miller A.B., & Day N.E. (1986). Screening for Cancer of the Uterine Cervix. IARC Sci. Publ. N° 76: 1-308. Lyon, International Agency for Research on Cancer

Halford J.A., Wright R.G., & Ditchmen E.J. (1999). Prospective study of PAPNET : review of 25656. Pap smears negative on manual screening and rapid rescreening. *Cytopathology* **10**: 317-324.

Hildesheim A., Schiffman M.H., & Gravitt P.E. (1994). Persistence of Type-Specific Human Papillomavirus Infection among Cytologically Normal Women. *J. Infect. Dis.* **169**: 235-240.

Ho G.Y.F., Burk R.D., Klein S., Kadish A.S., Chang C.J., Palan P., Basu J., Tachezy R., Lewis R., & Romney S. (1995). Persistent genital human papillomavirus infection as a risk factor for persistent

cervical dysplasia. *J. Natl. Cancer Inst.* **87**: 1365-1371.

Holowaty P., Miller A.B., Rohan T., & To T. (1999). Natural History of Dysplasia of the Uterine Cervix. *J. Natl. Cancer Inst.* **91**: 252-258.

Hopman E.H., Voorhorst F.J., Kenemans P., Meyer C.J.L.M., & Helmerhorst T.J.M. (1995). Observer agreement on interpreting colposcopic images of CIN. *Gynecol. Oncol.* **58**: 206-209.

Howell L.P., Davis R.L., Belk T.I., Agdigos R., & Lowe J. (1998). The AutoCyte preparation system for gynecologic cytology. *Acta Cytol.* **42**: 171-177.

Hutchinson M.L., Isenstein L.M., Goodman A., Hurley A., Douglas K.L., Mui K.K., Patten F.W., & Zahniser D.J. (1994). Homogeneous sampling accounts for the increased diagnostic accuracy using the thinprep processor. *Am. J. Clin. Pathol.* **101**: 215-219.

IARC (2005). Cervix Cancer Screening. IARC Handbooks of Cancer Prevention. **Vol. 10**. IARCPress, Lyon.

Iftner T. & Villa L.L. (2003). Chapter 12: Human papillomavirus technologies. *J. Natl. Cancer Inst. Monogr.* 80-88.

Irwig L., Glasziou P.P., Berry G., Chock C., Mock P., & Simpson J.M. (1994). Efficient Study Designs to Assess the Accuracy of Screening Tests. *Am. J. Epidemiol.* **140**: 759-769.

Jacobs M.V., Snijders P.J., Voorhorst F.J., Dillner J., Forslund O., Johansson B., von Knebel D.M., Meijer C.J.L.M., Nindl I., Pfister H., Stockfleth E., Strand A., Wadell G., & Walboomers J.M. (1999). Reliable high risk HPV DNA testing by polymerase chain reaction: and intermethod and intramethod comparison. *J. Clin. Pathol.* **52**: 498-503.

Jacobs M.V., Walboomers J.M., Snijders P.J., Voorhorst F.J., Verheijen R.H., Fransen-Daalmeijer N., & Meijer C.J. (2000). Distribution of 37 mucosotropic HPV types in women with cytologically normal cervical smears: the age-related patterns for high-risk and low-risk types. *Int. J. Cancer* **87**: 221-227.

Jain S., Tseng C.J., Horng S.G., Soong Y.K., & Pao C.C. (2001). Negative predictive value of human papillomavirus test following conization of the cervix uteri. *Gynecol. Oncol.* **82**: 177-180.

Jansen F.W., Trimboos J.B., Hermans J., & Fleuren G.J. (1994). Persistent cervical intraepithelial neoplasia after incomplete conization: predictive value of clinical and histological parameters. *Gynecol. Obstet. Invest.* **37**: 270-274.

Josefsson A.M., Livak K., & Gillensten U. (1999). Viral load of human papilloma virus 16 as a determinant for development of cervical carcinoma in situ; a nested case-control study. *J. Clin. Microbiol.* **37**: 490-496.

Josefsson A.M., Magnusson P.K.E., Ylitalo N., Sorensen P., Qwarforth-Tubbin P., Andersen P.K., Melbye M., & Adami H.-O. (2000). Viral load of human papilloma virus 16 as a determinant for development of cervical carcinoma in situ: a nested case-control. *Lancet* **355**: 2189-2193.

Kalliala I., Anttila A., Pukkala E., & Nieminen P. (2005). Risk of cervical and other cancers after treatment of cervical intraepithelial neoplasia: retrospective cohort study. *BMJ* **331**: 1183-1185.

Kanamori Y. & Kigawa J. (1998). Residual disease and presence of human papillomavirus after conization. *Oncology* **55**: 517-520.

Killough B.W., Clark A.H., & Garvin J.B. (1988). Correlation between cytodiagnosis and the presence of endocervical or squamous metaplastic cells in gynecologic smears. *Acta Cytol.* **32**: 758.

Kim C.J., Jeong J.K., Park M., Park T.S., Park T.C., Namkoong S.E., & Park J.S. (2003). HPV oligonucleotide microarray-based detection of HPV genotypes in cervical neoplastic lesions. *Gynecol. Oncol.* **89**: 210-217.

Kim J.J., Wright T.C., & Goldie S.J. (2002). Cost-effectiveness of alternative triage strategies for atypical squamous cells of undetermined significance. *JAMA* **287**: 2382-2390.

Kitchener H.C., Almonte M., Wheeler P., Desai M., Gilham C., Bailey A., Sargent A., & Peto J. (2006). HPV testing in routine cervical screening: cross sectional data from the ARTISTIC trial. *Br. J. Cancer* 56-61.

Kjellberg L., Wiklund F., Sjöberg I., Wadell G., Angstrom T., Dillner J., & Mahlick C.G. (1998). A population-based study for predicting cervical intraepithelial neoplasia. *Am. J. Obstet. Gynecol.* **179**: 1497-1502.

Klaes R. & Woerner S.M. (1999). Detection of high-risk cervical intraepithelial neoplasia and cervical cancer by amplification of transcripts derived from integrated papillomavirus oncogenes. *Cancer Res.* **59**: 6132-6136.

Kleter B., van Doorn L.J., Schrauwen L., Molijn A., Sastrowijoto S., ter Schegget J., Lindeman J., ter Harmsel B., Burger M., & Quint W. (1999). Development and clinical evaluation of a highly sensitive PCR-reverse hybridization line probe assay for detection and identification of anogenital human papillomavirus. *J. Clin. Microbiol.* **37**: 2508-2517.

Kleter B., van Doorn L.J., Schrauwen L., van Krimpen K., Burger M., ter Harmsel B., & Quint W. (1998). Novel short-fragment PCR assay for highly sensitive broad-spectrum detection of anogenital human papillomaviruses. *Am. J. Pathol.* **153**: 1731-1739.

Kok M.R. & Boon M.E. (1996). Consequences of neural network technology for cervical screening: increase of diagnostic consistency and increase of positive scores. *Cancer* **78**: 112-117.

Kok M.R., Boon M.E., Schreiner-Kok P.G., & Koss L.G. (2000). Cytological recognition of invasive squamous cancer of the uterine cervix: comparison of conventional light-microscopical screening and neural network-based screening. *Hum. Pathol.* **31**: 23-28.

Koliopoulos G., Martin-Hirsch P., Paraskevidis E., & Arbyn M. (2006). HPV testing versus cervical cytology for screening for cancer of the uterine cervix (Protocol). *The Cochrane Library* 1-9.

Koliopoulos G., Arbyn M., Martin-Hirsch P., Kyrgiou M., Prendiville W., & Paraskevidis E. (2007). Diagnostic accuracy of human papillomavirus testing in primary cervical screening: a systematic review and meta-analysis of non randomised studies. *Gynecol. Oncol.* **104**: 232-246.

Konya J., Veress G., Juhasz A., Szarka K., Sapy T., Hernadi Z., & Gergely L. (2000). Additional human papillomavirus types detected by the hybrid capture tube test among samples from women with cytological and colposcopic atypia. *J. Clin. Microbiol.* **38**: 408-411.

Kornegay J.R., Shepard A.P., Hankins C., Franco E.L., Lapointe N., Richardson H., & Coutlee F. (2001). Nonisotopic detection of human papillomavirus DNA in clinical specimens using a consensus PCR and a generic probe mix in an enzyme-linked immunosorbent assay format. *J. Clin. Microbiol.* **39**: 3530-3536.

Koss L.G., Sherman M.E., Cohen M.B., Anes A.R., Darragh T.M., Lemos L.B., McClellan B.J.,

METHODS FOR SCREENING AND DIAGNOSIS

Rosenthal D.L., Keyhani-Rofagha S., Schreiber K., & Valente P.T. (1997). Significant reduction in the rate of false-negative cervical smears with neural network-based technology (papnet testing system). *Hum. Pathol.* **28**: 1196-1203.

Kotaniemi-Talonen L., Nieminen P., Anttila A., & Hakama M. (2005). Routine cervical screening with primary HPV testing and cytology triage protocol in a randomised setting. *Br. J. Cancer* **93**: 862-867.

Kuhn L., Denny L., Pollack A., Lorincz A.T., Richart R.M., & Wright T.C. (2000). Human papillomavirus DNA testing for cervical cancer screening in low-resource settings. *J. Natl. Cancer Inst.* **92**: 818-825.

Kulasingam S.L., Hughes J.P., Kiviat N.B., Mao C., Weiss N.S., Kuypers J.M., & Koutsky L.A. (2002). Evaluation of human papillomavirus testing in primary screening cervical abnormalities. Comparison of sensitivity, specificity, and frequency of referral. *JAMA* **288**: 1749-1757.

Kurman R.J., Henson D.E., Herbst A.L., Noller K.L., Schiffman M.H., & National Cancer Institute (1994). Interim guidelines for management of abnormal cervical cytology. *JAMA* **271**: 1866-1869.

Linder J. & Zahniser D. (1997). The ThinPrep Pap test. A review of clinical studies. *Acta Cytol.* **41**: 30-38.

Lorincz A.T. (1997) Methods of DNA hybridisation and their clinical applicability to human papillomavirus detection. In: *New developments in cervical cancer screening and prevention* (eds Franco E.L. & Monsonego J.), Blackwell Science edn, pp. 325-337. Oxford.

Luff R.D. & et al (1992). The revised Bethesda system for reporting cervical/vaginal cytological diagnoses. Report of the 1991 Bethesda Workshop. *Acta Cytol.* **36**: 273-276.

Mann C.H., Steele J.C., Burton A., Bailey A.S., Etherington I.J., & Lusley D.M. (2001). LLETZ - Evidence of its efficacy against HPV infection. *Gynecol. Oncol.* **81**: 125-127.

Martin-Hirsch P., Koliopoulos G., & Paraskeva E. (2002). Is it now time to evaluate the true accuracy of cervical cytology screening? A review of the literature. *Eur. J. Gynaecol. Oncol.* **23**: 363-365.

Mayor S. (2003). NHS cervical screening programme to introduce liquid based cytology. *BMJ* **327**: 948-949.

McCrory D.C., Matchar D.B., Bastian L., Datta S., Hasselblad V., Hickey J., Myers E., & Nanda K. (1999). Evaluation of cervical cytology. AHCPR Publication **No. 99-E010**, 1-274. Rockville (MD), USA, AHCPR

McGoogan E. & Reith A. (1996). Would monolayers provide more representative samples and improved preparations for cervical screening ? *Acta Cytol.* **40**: 107-119.

Meijer C.J., Snijders P.J., & Castle P.E. (2006). Clinical utility of HPV genotyping. *Gynecol. Oncol.* **103**: 12-17.

Michelow P.M., Hlongwane N.F., & Leiman G. (1997). Simulation of primary cervical cancer screening by the PAPNET system in an unscreened, high-risk community. *Acta Cytol.* **41**: 88-92.

Mitchell H. & Medley G. (1991). Longitudinal study of women with negative cervical smears according to endocervical status. *Lancet* **337**: 265-267.

- Mitchell M.F., Schottenfeld D., Tortolero-Luna G., Cantor S.B., & Richards Kortum R. (1998). Colposcopy for the diagnosis of squamous intraepithelial lesions : a meta-analysis. *Obstet. Gynecol.* **91**: 626-631.
- Moberg M., Gustavsson I., & Gyllensten U. (2003). Real-time PCR-based system for simultaneous quantification of human papillomavirus types associated with high risk of cervical cancer. *J. Clin. Microbiol.* **41**: 3221-3228.
- Moberg M., Gustavsson I., & Gyllensten U. (2004). Type-specific associations of human papillomavirus load with risk of developing cervical carcinoma in situ. *Int. J. Cancer* **112**: 854-859.
- Molden T., Kraus I., Karlsen F., Skomedal H., Nygard J.F., & Hagmar B. (2005a). Comparison of human papillomavirus messenger RNA and DNA detection: a cross-sectional study of 4,136 women >30 years of age with a 2-year follow-up of high-grade squamous intraepithelial lesion. *Cancer Epidemiol. Biomarkers Prev.* **14**: 367-372.
- Molden T., Nygard J.F., Kraus I., Karlsen F., Nygard M., Skare G.B., Skomedal H., Thoresen S.O., & Hagmar B. (2005b). Predicting CIN2+ when detecting HPV mRNA and DNA by PreTect HPV-proofer and consensus PCR: A 2-year follow-up of women with ASCUS or LSIL pap smear. *Int. J. Cancer* **114**: 973-976.
- Morrison A.S. (1992). Screening in Chronic Disease **2nd**, 1-254. Oxford University Press, Inc.
- Moss S.M., Gray A., Legood R., & Henstock E. (2003). Evaluation of HPV/LBC. Cervical screening pilot studies. First report to the Department of Health on evaluation of LBC (December 2002). Institute of Cancer Research (Sutton); Institute of Health Sciences (Oxford): 1-96.
- Myers E.R., McCrory D.C., Subramanian S., McCall N., Nanda K., Datta S., & et al (2000). Setting the targets for a better cervical screening test: characteristics of a cost-effective test for cervical neoplasia screening. *Obstet. Gynecol.* **96**: 645-652.
- Nagai Y., Maehama T., Asato T., & Kanazawa K. (2000). Persistence of human papillomavirus infection after therapeutic conization for CIN 3: is it an alarm for disease recurrence? *Gynecol. Oncol.* **79**: 294-299.
- Nakagawa S., Yoshikawa H., Yasugi T., Kimura M., Kawana K., Matsumoto K., Yamada M., Onda T., & Taketani Y. (2000). Ubiquitous presence of E6 and E7 transcripts in human papillomavirus-positive cervical carcinomas regardless of its type. *J. Med. Virol.* **62**: 251-258.
- Nanda K., McCrory D.C., Myers E.R., Bastian L.A., Hasselblad V., Hickey J.D., & Matchar D.B. (2000). Accuracy of the Papanicolaou Test in Screening for and Follow-up of Cervical Cytologic Abnormalities: A Systematic Review. *Ann. Intern. Med.* **132**: 810-819.
- National Cancer Institute (1989). The 1988 Bethesda System for Reporting Cervical/Vaginal Cytologic Diagnoses. *Acta Cytol.* **33**: 567-571.
- National Institute for Clinical Excellence (NICE) (2003). Guidance on the use of liquid-based Cytology for Cervical Screening (Technology Appraisal No. 69). National Health Services of the United Kingdom, London.
- NHSCSP (2000). Achievable standards, benchmarks for reporting, and criteria for evaluating cervical cytopathology. Second edition including revised performance indicators (2nd edition). NHSCSP **publication 1**, 1-36. Sheffield, NHS Cancer Screening Programmes
- Nieminen P., Hakama M., Viikki M., Tarkkanen J., & Anttila A. (2003). Prospective and randomised

public-health trial on neural network-assisted screening for cervical cancer in Finland: results of the first year. *Int. J. Cancer* **103**: 422-426.

Nieminen P., Kotaniemi L., Hakama M., Tarkkanen J., Martikainen J., Toivonen T., Ikkala J., Luostarinen T., & Anttila A. (2005). A randomised public-health trial on automation-assisted screening for cervical cancer in Finland: performance with 470,000 invitations. *Int. J. Cancer* **115**: 307-311.

Nieminen P., Vuorma S., Viikki M., Hakama M., & Anttila A. (2004). Comparison of HPV test versus conventional and automation-assisted Pap screening as potential screening tools for preventing cervical cancer. *BJOG* **111**: 842-848.

Nobbenhuis M.A., Meijer C.J., van den Brule A.J., Rozendaal L., Voorhorst F.J., Risse E.K., Verheijen R.H., & Helmerhorst T.J. (2001). Addition of high-risk HPV testing improves the current guidelines on follow-up after treatment for cervical intraepithelial neoplasia. *Br. J. Cancer* **84**: 796-801.

Nygard J.F., Sauer T., Nygard M., Skare G.B., & Thoresen S.O. (2004). CIN 2/3 and cervical cancer in an organised screening programme after an unsatisfactory or a normal Pap smear: a seven-year prospective study of the Norwegian population-based screening programme. *J. Med. Screen.* **11**: 70-76.

Oh Y.L., Shin K.J., Han J., & Kim D.S. (2001). Significance of high-risk human papillomavirus detection by polymerase chain reaction in primary cervical cancer screening. *Cytopathology* **12**: 75-83.

Ostor A.G. (1993). Natural history of cervical intraepithelial neoplasia: a critical review. *Int. J. Gynecol. Pathol.* **12**: 186-192.

Pagliusi S.R., Dillner J., Pawlita M., Quint W.G., Wheeler C.M., & Ferguson M. (2006). Chapter 23: International Standard reagents for harmonization of HPV serology and DNA assays-an update. *Vaccine* **24**: 193-200.

Papillo J.L., Lee K.R., & Manna E.A. (1992). Clinical Evaluation of the ThinPrep method for the preparation of nongynecologic material. *Acta Cytol.* **36**: 651-652.

Paraskevaïdis E., Arbyn M., Diakomanolis E., Martin-Hirsch P., Koliopoulos G., Makrydimas G., Tofoski J., & Roukos D. (2004). The role of HPV DNA testing in the follow-up period after treatment for CIN: a systematic review of the literature. *Cancer Treat. Rev.* **30**: 205-211.

Paraskevaïdis E., Koliopoulos G., Alamanos Y., Malamou-Mitsi V., Lolis E.D., & Kitchener H.C. (2001a). Human papillomavirus testing and the outcome of treatment for cervical intraepithelial neoplasia. *Obstet. Gynecol.* **98**: 833-836.

Paraskevaïdis E., Lolis E.D., Koliopoulos G., Alamanos Y., Fotiou S., & Kitchener H.C. (2000). Cervical intraepithelial neoplasia outcomes after large loop excision with clear margins. *Obstet. Gynecol.* **95**: 828-831.

Paraskevaïdis E., Malamou-Mitsi V., Koliopoulos G., Pappa L., Lolis E., Georgiou I., & Agnantis N.J. (2001b). Expanded cytological referral criteria for colposcopy in cervical screening: comparison with human papillomavirus testing. *Gynecol. Oncol.* **82**: 355-359.

Payne N., Chilcott J., & McGoogan E. (2000). Liquid-based cytology in cervical screening: a rapid and systematic review. *Health Technol. Assess.* **4**: 1-73.

Pepe M.S. (2003). The Statistical Evaluation of Medical Tests for Classification and Prediction.

Oxford University Press, Oxford.

Petry K.U., Menton S., Menton M., Loenen-Frosch F., de Carvalho G.H., Holz B., Schopp B., Garbrecht-Buettner S., Davies P., Boehmer G., van den A.E., & Iftner T. (2003). Inclusion of HPV testing in routine cervical cancer screening for women above 29 years in Germany: results for 8466 patients. *Br. J. Cancer* **88**: 1570-1577.

Peyton C.L., Gravitt P.E., Hunt W.C., Hundley R.S., Zhao M., Apple R.J., & Wheeler C.M. (2001). Determinants of genital human papillomavirus detection in a US population. *J. Infect. Dis.* **183**: 1554-1564.

Peyton C.L., Schiffman M.A., Lörincz A.T., Hunt W.C., Mielzynska I., Bratti C., Eaton S., Hildesheim A., Morera L.A., Rodriguez A.C., Herrero R., Sherman M.E., & Wheeler C.M. (1998). Comparison of PCR- and hybrid capture-based human papillomavirus detection systems using multiple cervical specimen collection strategies. *J. Clin. Microbiol.* **36**: 3248-3254.

Ponten J., Adami H.-O., Bergstrom R., Dillner J., Friberg L., Gustafsson L., Miller A.B., Parkin D.M., Sparen P., & Trichopoulos D. (1995). Strategies for global control of cervical cancer. *Int. J. Cancer* **60**: 1-26.

Pretorius R.G., Belinson J.L., Zhang W.H., Burchette R.J., Elson P., & Qiao Y.L. (2001). The colposcopic impression. Is it influenced by the colposcopist's knowledge of the findings on the referral Papanicolaou smear? *J. Reprod. Med.* **46**: 724-728.

Pretorius R.G., Kim R.J., Belinson J.L., Elson P., & Qiao Y.L. (2006). Inflation of sensitivity of cervical cancer screening tests secondary to correlated error in colposcopy. *J. Low Genit. Tract. Dis.* **10**: 5-9.

Pretorius R.G., Zhang W.H., Belinson J.L., Huang M.N., Wu L.Y., Zhang X., & Qiao Y.L. (2004). Colposcopically directed biopsy, random cervical biopsy, and endocervical curettage in the diagnosis of cervical intraepithelial neoplasia II or worse. *Am. J. Obstet. Gynecol.* **191**: 430-434.

PRISMATIC Project Management Team (1999). Assessment of automated primary screening on PAPNET of cervical smears in the PRISMATIC trial. *Lancet* **353**: 1381-1385.

Qu W., Jiang G., Cruz Y., Chang C.J., Ho G.Y., Klein R.S., & Burk R.D. (1997). PCR detection of human papillomavirus: comparison between MY09/MY11 and GP5+/GP6+ primer systems. *J. Clin. Microbiol.* **35**: 1304-1310.

Quint W.G., Pagliusi S.R., Lelie N., de Villiers E.M., & Wheeler C.M. (2006). Results of the first World Health Organization international collaborative study of detection of human papillomavirus DNA. *J. Clin. Microbiol.* **44**: 571-579.

Quint W.G., Scholte G., van Doorn L.J., Kleter B., Smits P.H., & Lindeman J. (2001). Comparative analysis of human papillomavirus infections in cervical scrapes and biopsy specimens by general SPF(10) PCR and HPV genotyping. *J. Pathol.* **194**: 51-58.

Raffle A.E., Alden B., & Mackenzie E.F.D. (1995). Detection rates for abnormal cervical smears: what are we screening for? *Lancet* **345**: 1469-1473.

Ransdell J.S., Davey D.D., & Zaleski S. (1997). Clinicopathologic correlation of the unsatisfactory Papanicolaou smear. *Cancer* **81**: 139-143.

Ratnam S., Franco E.L., & Ferenczy A. (2000). Human papillomavirus testing for primary screening of cervical cancer precursors. *Cancer Epidemiol. Biomarkers Prev.* **9**: 945-951.

Remmink A.J., Walboomers J.M., Helmerhorst T.J.M., Voorhorst F.J., Rozendaal L., Risse E.K.J., Meijer C.J.L.M., & Kenemans P. (1995). The presence of persistent high-risk HPV genotypes in dysplastic cervical lesions is associated with progressive disease: natural history up to 36 months. *Int. J. Cancer* **61**: 306-311.

Ronco G., Ghisetti V., Segnan N., Snijders P.J., Gillio-Tos A., Meijer C.J., Merletti F., & Franceschi S. (2005). Prevalence of human papillomavirus infection in women in Turin, Italy. *Eur J Cancer* **41**: 297-305.

Ronco G., Giorgi-Rossi P., Carozzi F., Dalla P.P., Del Mistro A., De Marco L., De Lillo M., Naldoni C., Pierotti P., Rizzolo R., Segnan N., Schincaglia P., Zorzi M., Confortini M., & Cuzick J. (2006a). Human papillomavirus testing and liquid-based cytology in primary screening of women younger than 35 years: results at recruitment for a randomised controlled trial. *Lancet Oncol.* **7**: 547-555.

Ronco G., Segnan N., Giorgi-Rossi P., Zappa M., Casadei G.P., Carozzi F., Dalla Palma P., Del Mistro A., Folicaldi S., Gillio-Tos A., Nardo G., Naldoni C., Schincaglia P., Zorzi P., Confortini M., & Cuzick J. (2006b). Human Papillomavirus testing and liquid-based cytology in primary cervical screening: results at recruitment from the New Technologies for Cervical Cancer randomized controlled trial. *J. Natl. Cancer Inst.* **98**: 765-774.

Ronco G., Vineis C., Montanari G., Orlassino R., Parisio F., Arnaud S., Berardengo E., Fabbri T., & Segnan N. (2003). Impact of the AutoPap (currently Focalpoint) primary screening system location guide use on interpretation time and diagnosis. *Cancer* **99**: 83-88.

Royston G. (1999). Commentary: trials versus models in appraising screening programmes. *BMJ* **318**: 360-361.

Rozendaal L., Walboomers J.M., van der Linden J.C., Voorhorst F.J., Kenemans P., Helmerhorst T.J., van Ballegooijen M., & Meijer C.J. (1996). PCR-based high-risk HPV test in cervical cancer screening gives objective risk assessment of women with cytomorphologically normal cervical smears. *Int. J. Cancer* **68**: 766-769.

Rubio C.A. (1977). The false negative smear : II. The trapping effect of collecting instruments. *Obstet. Gynecol.* **49**: 576-580.

Sankaranarayanan R., Chatterji R., Shastri S.S., Basu P., Mahé C., Muwonge R., Seigneurin D., Somanathan T., Roy C., Kelkar R., Chinoy R., Dinshaw K., Mandal R., Amin G., Goswami S., Pal S., Patil S., Dhakad N., Frappart L., & Fontaniere B. (2004). Accuracy of human papillomavirus testing in primary screening of cervical neoplasia: results from a multicentre study in India. *Int. J. Cancer* **112**: 341-347.

Sankaranarayanan R., Nene B.M., Dinshaw K.A., Mahe C., Jayant K., Shastri S.S., Malvi S.G., Chinoy R., Kelkar R., Budukh A.M., Keskar V., Rajeshwarker R., Muwonge R., Kane S., & Parkin D.M. (2005). A cluster randomized controlled trial of visual, cytology and human papillomavirus screening for cancer of the cervix in rural India. *Int. J. Cancer* **116**: 617-623.

Sarian L.O., Derchain S.F., Naud P., Roteli-Martins C., Longatto-Filho A., Tatti S., Branca M., Erzen M., Serpa-Hammes L., Matos J., Gontijo R.C., Braganca J.F., Lima T.P., Maeda M.Y., Lorincz A., Dores G.B., Costa S., Syrjänen S.M., & Syrjänen K.J. (2005). Evaluation of visual inspection with acetic acid (VIA), Lugol's iodine (VILI), cervical cytology and HPV testing as cervical screening tools in Latin America. *J. Med. Screen.* **12**: 142-149.

Sasieni P.D., Cuzick J., Lynch-Farmery E.L., & National Co-ordinating Network for Cervical Screening Working Group (1996). Estimating the efficacy of screening by auditing smear histories of women

with and without cervical cancer. *Br. J. Cancer* **73**: 1001-1005.

Sawaya G.F. (2005). A 21-year-old woman with atypical squamous cells of undetermined significance. *JAMA* **294**: 2210-2218.

Schatzkin A., Connor R.J., Taylor P.R., & Bunnag B. (1987). Comparing new and old screening tests when a reference procedure cannot be performed on all screenees. Example of automated cytometry for early detection of cervical cancer. *Am. J. Epidemiol.* **125**: 672-678.

Schiffman M.A. & Adriaenza M.E. (2000). ASCUS-LSIL Triage Study. Design, methods and characteristics of trial participants. *Acta Cytol.* **44**: 726-742.

Schiffman M.A., Herrero R., Desalle R., Hildesheim A., Wacholder S., Rodriguez A.C., Bratti M.C., Sherman M.E., Morales J., Guillen D., Alfaro M., Hutchinson M., Wright T.C., Solomon D., Chen Z., Schussler J., Castle P.E., & Burk R.D. (2005a). The carcinogenicity of human papillomavirus types reflects viral evolution. *Virology* **337**: 76-84.

Schiffman M.A., Herrero R., Hildesheim A., Sherman M.E., Bratti M., Wacholder S., Alfaro M., Hutchinson M., Morales J., Greenberg M.D., & Lorincz A.T. (2000). HPV DNA testing in cervical cancer screening. Results from women in a high-risk province of Costa Rica. *JAMA* **283**: 87-93.

Schiffman M.A., Khan M.J., Solomon D., Herrero R., Wacholder S., Hildesheim A., Rodriguez A.C., Bratti M.C., Wheeler C.M., & Burk R.D. (2005b). A study of the impact of adding HPV types to cervical cancer screening and triage tests. *J. Natl. Cancer Inst.* **97**: 147-150.

Schmitt M., Bravo I.G., Snijders P.J., Gissmann L., Pawlita M., & Waterboer T. (2006). Bead-based multiplex genotyping of human papillomaviruses. *J. Clin. Microbiol.* **44**: 504-512.

Schneider A., Hoyer H., Lotz B., Leistritz S., Kuhne-Heid R., Nindl I., Muller B., Haerting J., & Durst M. (2000). Screening for high-grade cervical intra-epithelial neoplasia and cancer by testing for high-risk HPV, routine cytology or colposcopy. *Int. J. Cancer* **89**: 529-534.

Scottish Cervical Screening Programme (2002). Steering group report on the feasibility of introducing liquid based cytology. 1-16. Scotland

Sherlaw-Johnson C., Gallivan S., & Jenkins D. (1999). Withdrawing low risk women from cervical screening programmes: mathematical modelling study. *BMJ* **318**: 356-361.

Sherman M.E., Lorincz A.T., Scott D.R., Wacholder S., Castle P.E., Glass A.G., Mielzynska-Lohnas I., Rush B.B., & Schiffman M.A. (2003). Baseline Cytology, Human Papillomavirus Testing, and Risk for Cervical Neoplasia: A 10-Year Cohort Analysis. *J. Natl. Cancer Inst.* **95**: 46-52.

Sherman M.E., Schiffman M.A., & Cox J.T. (2002). Effects of age and human papilloma viral load on colposcopy triage: data from the randomised atypical squamous cells of undetermined significance/low-grade intraepithelial lesion triage study (ALTS). *J. Natl. Cancer Inst.* **94**: 102-107.

Sherman M.E., Schiffman M.H., Lorincz A.T., Herrero R., Hutchinson M.L., Bratti C., Zahniser D., Morales J., Hildesheim A., Helgesen K., Kelly D., Alfaro M., Mena F., Balmaceda I., Mango L., & Greenberg M. (1997). Cervical specimens collected in liquid buffer are suitable for both cytologic screening and ancillary human papillomavirus testing. *Cancer* **81**: 89-97.

Siebers A.G., de Leeuw H., Verbeek A.L., & Hanselaar A.G. (2003). Prevalence of squamous abnormalities in women with a recent smear without endocervical cells is lower as compared to women with smears with endocervical cells. *Cytopathology* **14**: 58-65.

Smits H.L., van Gemen B., Schukink R., Van der Velden J., Tjong A.H., Jebbink M.F., & ter Schegget J. (1995). Application of the NASBA nucleic acid amplification method for the detection of human papillomavirus type 16 E6-E7 transcripts. *J. Virol. Methods* **54**: 75-81.

Snijders P.J., van den Brule A.J., & Meijer C.J.L.M. (2003). The clinical relevance of human papillomavirus testing : relationship between analytical and clinical sensitivity. *J. Pathol.* **201**: 1-6.

Solomon D., Davey D., Kurman R., Moriarty A., O'Connor D., Prey M., Raab S., Sherman M.E., Wilbur D., Wright T.C., & Young N. (2002). The 2001 Bethesda System: terminology for reporting results of cervical cytology. *JAMA* **287**: 2114-2119.

Solomon D, Nayar R. (2004). The Bethesda system for reporting cervical cytology: definitions, criteria and explanatory notes, 2nd edn. Springer, New York.

Solomon D., Schiffman M.A., & Tarone B. (2001). Comparison of three management strategies for patients with atypical squamous cells of undetermined significance (ASCUS): baseline results from a randomized trial. *J. Natl. Cancer Inst.* **93**: 293-299.

Sotlar K., Selinka H.C., Menton M., Kandolf R., & Bultmann B. (1998). Detection of human papillomavirus type 16 E6/E7 oncogene transcripts in dysplastic and nondysplastic cervical scrapes by nested RT-PCR. *Gynecol. Oncol.* **69**: 114-121.

Soutter W.P., de Barros Lopes A., Fletcher A., Monaghan J.M., Duncan I.D., Paraskevaidis E., & Kitchener H.C. (1997). Invasive cervical cancer after conservative therapy for cervical intraepithelial neoplasia [see comments]. *Lancet* **349**: 978-980.

Soutter W.P., Sasieni P., & Panoskaltsis T. (2005). Long-term risk of invasive cervical cancer after treatment of squamous cervical intraepithelial neoplasia. *Int. J. Cancer* **118**: 2048-2055.

Studeman KD, Ioffe OB, Puzkiewicz J, Sauvegeot J, Henry MR. (2003). Effect of cellularity on the sensitivity of detecting squamous lesions in liquid-based cervical cytology. *Acta Cytol.* **47**:605-10.

Swan D.C., Tucker R.A., Holloway B.P., & Icenogle J.P. (1997). A sensitive, type-specific, fluorogenic probe assay for detection of human papillomavirus DNA. *J. Clin. Microbiol.* **35**: 886-891.

Taylor S., Kuhn L., Dupree W., Denny L., De Souza M., & Wright T.C., Jr. (2006). Direct comparison of liquid-based and conventional cytology in a South African screening trial. *Int. J. Cancer* **95**:957-962.

Tucker R.A., Unger E.R., Holloway B.P., & Swan D.C. (2001). Real-time PCR-based fluorescent assay for quantitation of human papillomavirus types 6, 11, 16, and 18. *Mol. Diagn.* **6**: 39-47.

van Ballegooijen M., van den Akker van Marle M.E., Patnick J., Lynge E., Arbyn M., Anttila A., Ronco G., & Habbema D.F. (2000). Overview of important cervical cancer screening process values in EU-countries, and tentative predictions of the corresponding effectiveness and cost-effectiveness. *Eur J Cancer* **36**: 2177-2188.

van Ballegooijen M., van den Akker van Marle M.E., Warmerdam P.G., Meijer C.J., Walboomers J.M., & Habbema J.D. (1997). Present evidence on the value of HPV testing for cervical cancer screening: a model-based exploration of the (cost-)effectiveness. *Br. J. Cancer* **76**: 651-657.

van den Akker van Marle M.E., van Ballegooijen M., Rozendaal L., Meijer C.J., & Habbema J.D. (2003). Extended duration of the detectable stage by adding HPV test in cervical cancer screening. *Br. J. Cancer* **89**: 1830-1833.

van den Brule A.J., Pol R., Fransen-Daalmeijer N., Schouls L.M., Meijer C.J., & Snijders P.J. (2002).

GP5+/6+ PCR followed by reverse line blot analysis enables rapid and high-throughput identification of human papillomavirus genotypes. *J. Clin. Microbiol.* **40**: 779-787.

van der Graaf Y., Molijn A., Doornwaard H., Quint W., van Doorn L.J., & van den Tweel J. (2002). Human papillomavirus and the long-term risk of cervical neoplasia. *Am. J. Epidemiol.* **156**: 158-164.

van Doorn L.J., Quint W., Kleter B., Molijn A., Colau B., Martin M.T., Kravang I., Torrez-Martinez N., Peyton C.L., & Wheeler C.M. (2002). Genotyping of human papillomavirus in liquid cytology cervical specimens by the PGMV line blot assay and the SPF(10) line probe assay. *J. Clin. Microbiol.* **40**: 979-983.

van Doornum G.J., Prins M., Juffermans L.H., Hooykaas C., van den Hoek J.A., Coutinho R.A., & Quint W.G. (1994). Regional distribution and incidence of HPV infections among heterosexual men and women with multiple partners: A prospective study. *Genitourin. Med.* **70**: 240-246.

van Oortmarssen G.J. & Habbema J.D. (1991). Epidemiological evidence for age-dependent regression of pre- invasive cervical cancer. *Br. J. Cancer* **64**: 559-565.

van Oortmarssen G.J., Habbema J.D.F., & van Ballegooijen M. (1992). Predicting mortality from cervical cancer after negative smear test results. *BMJ* **305**: 449-451.

Vernon S.D., Unger E.R., & Williams D. (2000). Comparison of human papillomavirus detection and typing by cycle sequencing, line blotting, and hybrid capture. *J. Clin. Microbiol.* **38**: 651-655.

von Knebel D.M. (2002). New markers for cervical dysplasia to visualise the genomic chaos created by aberrant oncogenic papillomavirus infections. *Eur J Cancer* **38**: 2229-2242.

Vooijs P.G., Elias A., van der Graaf Y., & Veling S. (1985). Relationship between the diagnosis of epithelial abnormalities and the composition of cervical smears. *Acta Cytol.* **29**: 323-328.

Wallace J., Woda B.A., & Pihan G. (2005). Facile, comprehensive, high-throughput genotyping of human genital papillomaviruses using spectrally addressable liquid bead microarrays. *J. Mol. Diagn.* **7**: 72-80.

WHO (2006). Comprehensive cervical cancer control: A guide to essential practice. WHO Press, Geneva

Wilbur D.C., Bonfiglio T.A., Rutkowski M.A., Atkison K.M., Richart R.M., Lee J.S., & Patten S.F., Jr. (1996). Sensitivity of the AutoPap 300 QC System for cervical cytologic abnormalities. Biopsy data confirmation. *Acta Cytol.* **40**: 127-132.

Wilson J.D., French R., Branch T., & Sutton J. (1999). Inadequate cervical cytology - the need to audit individual smear takers' inadequate rates. *Cytopathology* **10**: 107-111.

Wilson J.M.G. & Jungner G. (1968). Principles and practice of screening for disease. Public Health Papers 34. Geneva, World Health Organisation

Wilson S.H. & Johnson J. (1992). An audit of cervical cancer deaths in Nottingham. *Cytopathology* **3**: 79-83.

Woodman C.B., Collins S., Winter H., Bailey A., Ellis J., Prior P., Yates M., & Rollason T.P. (2001). Natural history of cervical human papillomavirus infection in young women: a longitudinal cohort study. *Lancet* **357**: 1831-1836.

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Wright J.D., Rader J.S., Davila R., Powell M.A., Mutch D.G., Gao F., & Gibb R.K. (2006). Human papillomavirus triage for young women with atypical squamous cells of undetermined significance. *Obstet. Gynecol.* **107**: 822-829.

Ylitalo N., Sorensen P., Josefsson A.M., Magnusson P.K.E., Andersen P.K., Ponten J., Adami H.-O., Gyllenstein U.B., & Melbye M. (2000). Consistent high viral load of human papillomavirus 16 and risk of cervical carcinoma in situ: a nested case-control study. *Lancet* **355**: 2194-2198.

Zielinski G.D., Bais A.G., Helmerhorst T.J., Verheijen R.H., De Schipper F.A., Snijders P.J., Voorhorst F.J., van Kemenade F.J., Rozendaal L., & Meijer C.J. (2004). HPV testing and monitoring of women after treatment of CIN 3: review of the literature and meta-analysis. *Obstet. Gynecol. Surv.* **59**: 543-553.

Zielinski G.D., Rozendaal L., Voorhorst F.J., Berkhof J., Snijders P.J., Risse E.J., Ronsink A.P., De Schipper F.A., & Meijer C.J.L.M. (2003). HPV testing can reduce the number of follow-up visits in women treated for cervical intraepithelial neoplasia grade 3. *Gynecol. Oncol.* **91**: 67-73.

zur Hausen H. (2002). Papillomaviruses and cancer: from basic studies to clinical application. *Nat. Rev. Cancer* **2**: 342-350.

Annex 1

Collection of cellular material of the uterine cervix

Preparation of an adequate Pap smear

1.1 Introduction

The correct sampling of the cervix with appropriate equipment contributes significantly to the diagnostic value of the Pap test (Buntinx *et al.*, 1992; Arbyn & Flemish Working Party Sampling, 2000; BSCC, 2003). Unsatisfactory samples are an important cause of false negative and false positive results. This guideline aims to bring together good practice in different countries across Europe, but it is recognized that there may be minor variations from recommendations of local and national programmes.

1.2 Facilities

The cervical screening programme will invite well women. It is important that women are satisfied with the service offered to them, or they will not return for rescreening or follow up tests. Before the smear is even taken, the environment for the taking of the smear should be suitable. There should be privacy, warmth and a relaxed atmosphere. The woman must be comfortable, and there must be an adjustable spotlight for the smear taker to visualise the cervix before taking the smear.

The equipment required for taking the sample should be available before beginning the examination to minimise the time the woman spends in what some consider to be an embarrassing position. The equipment that should be available will include gloves, a range of specula, sampling devices, slides, fixative, pencil and slide carrier for conventional smears or vials and a ballpoint pen where liquid-based cytology (LBC) is used. Special care should be taken to keep the interval between taking the sample and fixing it as short as possible. The top should already be removed from the fixative dropper bottle or aerosol can, and the can should be checked to ensure it is not blocked or empty. Waste disposal and sterilisation facilities will be required for when the examination is concluded.

In addition there should be leaflets available to give the woman information on a variety of issues that she might raise. The test request form should be properly completed.

Contra-indications for cervical screening cytology: total hysterectomy, cervical amputation (if the surgery was performed for a cervical lesion, a vaginal smear should be performed at the recommended frequency) and the presence of a suspect, macroscopically visible lesion in the area of the cervix. In the latter case, the woman must be referred for colposcopic examination and/or biopsy.

Factors adversely affecting the quality of a cell sample

- menstruation, blood loss, breakthrough bleeding
- vaginal inflammation/infection
- sexual intercourse within 24 hours
- severe genital atrophy (menopause)
- pregnancy, post-partum period and lactation
- physical manipulation or chemical irritation such as: preceding digital vaginal examination, disinfectant cream or liquid, lubricating jelly, vaginal medication, vaginal douche or spermicidal jelly (less than 24 hours before), prior colposcopy with acetic acid (less than 24 hours before), previous smear (less than 3 weeks before), cervical surgery (less than 3 months before)
- radiotherapy

It is essential to know these factors and reduce their effect to a minimum. The quality of the preparations may be poor in pregnancy and the early post-partum period due to reactive inflammatory changes. Therefore, taking a smear should be postponed for pregnant women with negative screening histories until 6-8 weeks after delivery unless the last smear was more than 3 years ago and / or compliance for screening is considered likely to be poor. If a previous smear was abnormal and in the interim the woman becomes pregnant then the follow-up smear should not be delayed. All relevant clinical information must be recorded on the request form.

1.3 Preparing to take the sample

Explain to the woman the aim of taking the sample and what to expect; and give reassurance. Ask about her general health and whether she has any symptoms such as irregular bleeding or discharge. The date of the last menstrual period or of a recent pregnancy should be noted. Follow any local consent protocols. Inform her that sometimes the examination has to be repeated within 3 to 6 months, if the smear was not of satisfactory quality. Make a clear arrangement about how the woman will be notified of the laboratory result.

For conventional smears, label the slide or slides clearly in pencil on the frosted end with the woman's identification data (including at least two parameters such as name, number, date of birth). Other methods of marking may be lost during processing of the slide. For LBC, label the vial with the same information using a ballpoint pen.

Ensure that the woman is lying comfortably on the examination couch in the dorsal or lateral position and position the light source so as to visualise the cervix clearly. Avoid taking a swab before the cervical sample.

Select the largest speculum that can be inserted comfortably and bring to body temperature by warming it in the gloved hand or in tepid water. Insert the speculum along the axis of the introitus and, when half way up the vagina, rotate 90° and open when fully inserted. Lubricants are not usually necessary. If required a little tepid water or a small amount of water-soluble lubricant may be used but this must not contaminate the surface of the cervix as this impairs the sample quality. Bring the cervix into view by gentle movement of the speculum encouraging the woman to relax. If this proves difficult, digital examination taking care not to disturb the surface of the cervix, or change in position may be beneficial. The appearance of the cervix should be noted. All those taking samples should be taught to recognise the various normal and abnormal appearances of the cervix and suspicious symptoms. Do not routinely clean the cervix or take a swab before taking the sample.

1.4 Sampling the transformation zone

The precursors of cervical cancer arise mainly in the transformation zone (TZ) between the ectocervical multilayer squamous epithelium and the endocervical columnar epithelium (Burghardt, 1970; Boon & Suurmeijer, 1993; Burghardt *et al.*, 1998). Therefore, it is important that cell material be sampled primarily from this zone. The presence of metaplastic squamous cells and endocervical cells, in addition to squamous cells, indicates that the transformation zone has been sampled but

cannot provide assurance that its full circumference has been sampled. In the past, absence of an endocervical component was considered as a reason to repeat the smear (Vooijs *et al.*, 1985). However, longitudinal studies have shown that women with a previous negative smear lacking endocervical cells (EC-) are not at higher risk for future cervical lesion compared to women with a negative EC+ smear (Bos *et al.*, 2001; Mitchell, 2001; Siebers *et al.*, 2003). Nevertheless, the presence of endocervical and / or metaplastic cells indicates that the target zone has been sampled.

1.4.1 Sampling devices

Cervical screening always requires an endocervical and an ectocervical sample, taken with the appropriate instruments. Sampling the transformation zone may be carried out using wooden or plastic spatulae of various types. Spatulae with extended tips, brooms and brushes are recommended sampling instruments (Buntinx & Brouwers, 1996; Martin-Hirsch *et al.*, 1999). We distinguish two possible ends in the spatula: Ayre (lower part in Fig. 1a) and extended tip or Aylesbury end (upper part of Fig. 1a). Use of cotton tip applicators is not advised.

Three methods are recommended.

- Cervical broom (Cervex-Brush, Rovers, Oss, The Netherlands) (Fig. 1c)
- Combination of a spatula (Fig. 1a) for the ectocervical sample and the endocervical brush (Fig. 1b) for the endocervical sample.
- Extended tip spatula alone (Fig. 1a, upper end).

An endocervical brush should never be used alone.

The cervical broom is best if the woman is pregnant or has a cervix that bleeds easily. The combination method, including the endocervical brush, is best if the squamo-columnar junction is high (often post menopausal), after cervical surgery or if there is extensive ectropion of the columnar epithelium. The endocervical brush should never be used alone. In the UK, one sample with an extended tip spatula is the recommended first choice (BSCC, 2003).

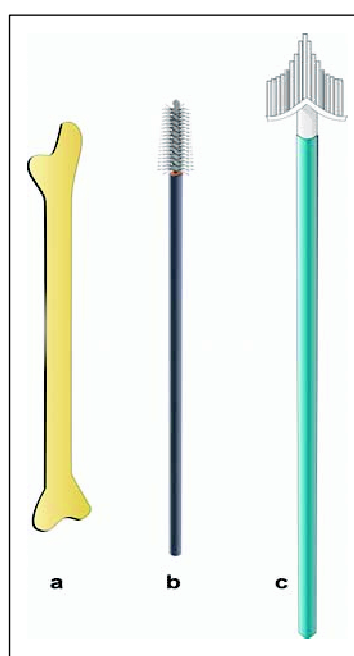


Fig. 1. Sampling devices: a) combined spatula with an *Aylesbury* end (extended tip) above and an *Ayre* end (below); b) endocervical brush; c) cervical broom

1.4.2 Sampling and preparing a conventional smear

1.4.2.1 Cervical broom

Endocervical cells and ectocervical cells are sampled simultaneously - the long bristles pick up endocervical cells while the short bristles collect ectocervical cells and are bevelled to collect cells when rotated in a clockwise direction only.

- The long bristles are positioned endocervically (Fig. 2).
- Rotate the brush five times over 360° with gentle pressure by rolling the handle clockwise between thumb and forefinger.
- Sweep the broom lengthwise along the slide, turn over and repeat for the other side.
- Fix immediately

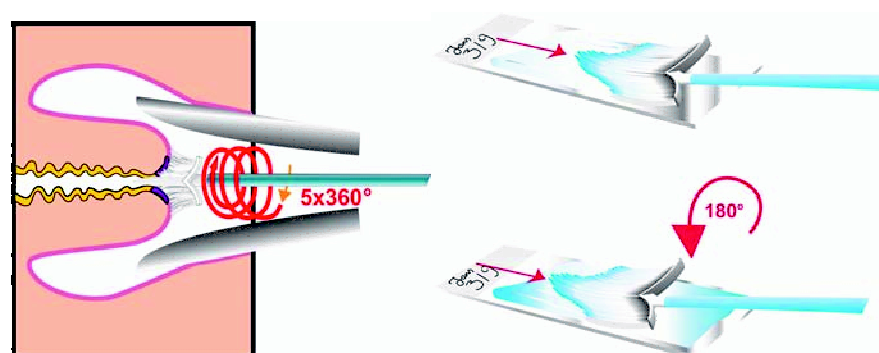


Fig. 2. Cervical broom: sampling and spreading the sample on the slide

The fixative of choice is 95% ethyl alcohol but other appropriate fixatives may be used. The smear should be flooded with fixative from a dropper bottle (Fig. 3a), placed immediately in a container of fixative that covers the whole of the cellular area of the slide or sprayed with an aerosol fixative (Fig. 3b). The slide should be fixed for at least 10 minutes. It should be removed from the fixative and placed dry in a slide box for transportation.

If spray fixation is used, the specimen should be fixed immediately by spraying at a right angle from a distance of 20 cm (Fig. 3b). If closer, the cells are blown away or frozen, if on a slant, the material is blown into aggregates. Droplet formation should be avoided by not using too much fixative. The BSCC guidelines recommend placing the slide on a flat surface for spray fixation, to avoid uneven fixation (BSCC, 2003; NHSCSP, 2006). Very fast fixation, within a few seconds, is essential to prevent drying artefacts.

It is critical that smears are fixed immediately to prevent partial air-drying, which will distort cellular detail. It should be noted that smears from postmenopausal women and blood-stained smears dry very rapidly.

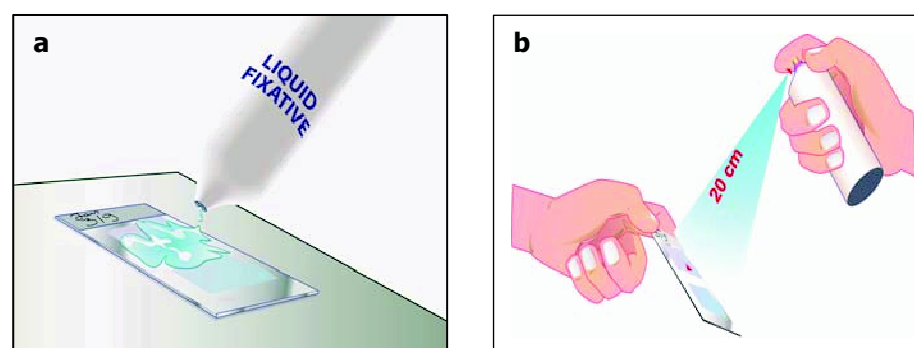


Fig. 3. Fixation of the smear by flooding with fixative from a dropper bottle (a: left) or by spraying (b: right).

1.4.2.2 Combination of spatula and endocervical brush

Spatula sampling

- Use the end of the spatula that is most appropriate to the anatomy of the portio. For nullipara, this is usually the Aylesbury end, for multipara the broader Ayre end. The pointed end of the spatula should be inserted into the cervical os until the inner curved surface is applied to the cervical surface.
- Rotate the spatula through more than one complete turn while maintaining firm contact with the cervix. When turning clockwise, stop at the 9 'o clock position; or when turning anti-clockwise stop at the 3 'o clock position, so that the scraped material remains on the upper side when the spatula is in the horizontal position (NCCLS, 1994).
- The tip scrapes the os while the less protruding part scrapes the surface of the portio. Take special care to scrape the squamocolumnar junction as fully as possible. If there is extensive ectropion, scrape the outer part of the portio separately using the blunt end of the Ayre spatula.
- Place the spatula on a rack and proceed without delay to take the brush sample. The danger of drying out is slightly less if the cell material and mucus remain in contact with the sampling device.

Endocervical brush sampling

- Insert the endocervical brush for two thirds into the endocervical canal, so that the lower bristles are still visible, and rotate gently 90 to 180°.

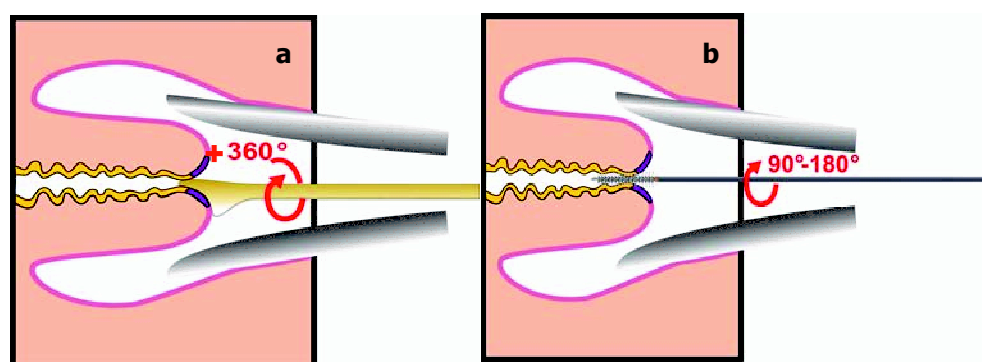


Fig. 4. Sampling of cellular material using the spatula (a: left) and the endocervical brush (b: right).

Transfer of cellular material onto the glass slide

Roll (not wipe) the endocervical brush immediately over the outer third of the slide in the opposite direction from which it was collected by twirling the handle (Fig. 5a). Do the rolling in a single movement (not in a zigzag) and without pressure, in order to obtain a thin and even smear.

Then spread the material from the spatula as quickly as possible onto the central third (Fig. 5b). Use firm longitudinal sweeps ensuring that material from both sides of the spatula is removed.

An alternative is to transfer the brush material lengthwise over the first half length and the spatula over the other half length of the slide (Fig. 5c & d).

Fix immediately using one of the methods described above. Endocervical cells dry very quickly and a drop of fixative spread on the slide before spreading the cellular sample may aid rapid fixation (BSCC, 2003).

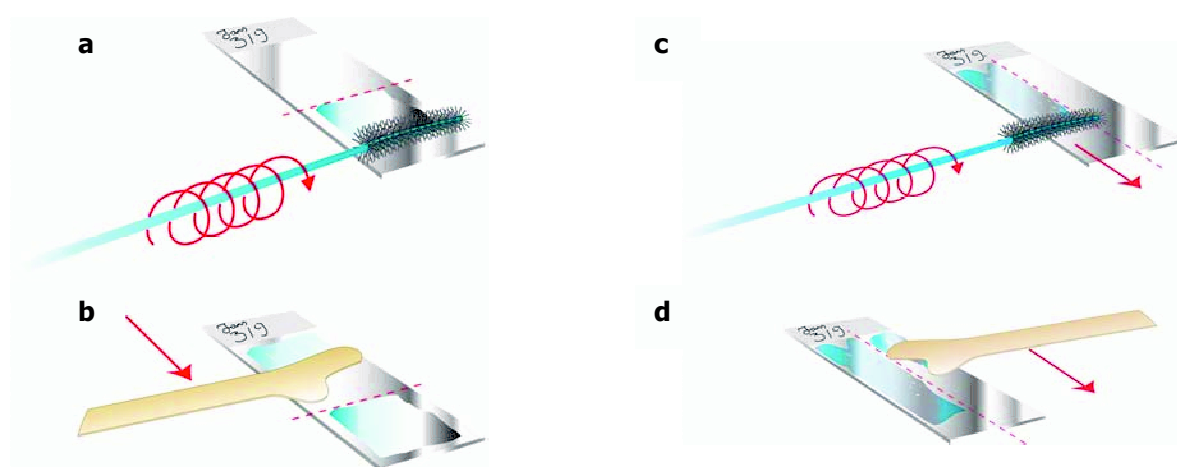


Fig. 5. Transfer of cellular material from the sampling device onto a glass slide

For inexperienced smear-takers, it can be difficult to spread the two samples on one slide and to fix both specimens adequately before the first sample dries. In this situation, it may be easier to spread the endocervical brush sample and the spatula sample over two slides. In that case, first fix the spatula sample, before proceeding to endocervical sampling.

1.4.2.3 Sampling with the extended tip spatula alone

Finally a third option is to collect the cells from the endocervix and exocervix with an extended tip spatula alone (Fig. 5a) and to spread one side of the spatula over one half length and the other side over the other half length (Fig. 5d). This option is the first choice in the UK (BSCC, 2003; NHSCSP, 2006).

1.4.3 Preparing a liquid-based cytology sample

A LBC sample is collected from the cervix in the same way as for a conventional smear, but only plastic sampling devices may be used. The manufacturers' instructions for collecting the sample must be followed. A broom-type sampling device or endocervical brush/plastic spatula combination

(with detachable head(s)) is recommended for the BD SurePath™ System (BD Diagnostics, Diagnostic Systems-TriPath USA). The protocol for rinsing the sample into the vial of collection fluid or detaching the head of the sampling device and placing it into the preservative fluid of the collection vial depends on the methodology used and should be confirmed against the manufacturer's instructions. In both instances the lid should be removed from the vial before the sampling procedure begins. Procedures for other liquid-based methods should be adapted according to the manufacturers' instructions.

For the ThinPrep system, the broom should be pressed vigorously against the bottom of the vial 15-20 times to remove all the cellular material (Fig. 6a). Before discarding the broom, the bristles should be inspected and the procedure for rinsing in the vial repeated if any residual material is seen.

For BD SurePath™ samples, the head of the sampling device is detached from the handle and placed into a vial of BD SurePath™ Preservative Fluid (Fig. 6b). Place the lid on the vial and tighten.

The lid of the vial should be firmly closed to prevent leakage during transportation. The ThinPrep Preservacyt vial has torque lines to facilitate correct sealing. Overtightening of the lid should be avoided since this may impede functioning of the T3000 automated ThinPrep processor.

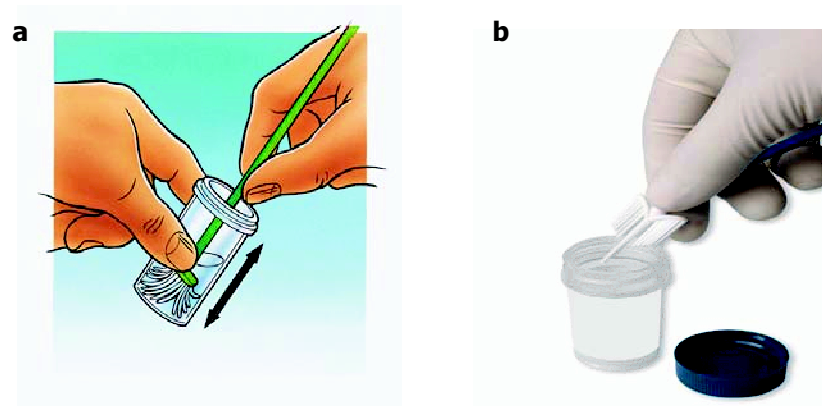


Fig. 6. Left (a): The broom is pressed multiple times vigorously against the bottom of the vial (ThinPrep sample). Image reprinted with permission from Cytyc Europe.

Right (b): The head of the sample collection device is detached and dropped into the preservative fluid of the BD SurePath® Collection vial. Image reprinted with permission of BD Diagnostics-Diagnostic Systems, TriPath.

1.4.4 Completing sampling

Removing the speculum from the vagina

In view of the importance of fixing the slides quickly, the speculum may be left in place, having explained to the woman before the procedure the reason for doing so, until the slides have been prepared and fixed. The speculum should be withdrawn gently with the blades apart until the cervix is no longer between them. The speculum should then be allowed to close as it is withdrawn completely (NHSCSP, 2006).

Complete the request form

After completing the procedure, the request form should be fully filled in with the woman's surname, forename, date of birth and other identifying features clearly written. The number of slides or sampling technique, date of last menstrual period or recent pregnancy, and clinical observations such as irregular bleeding or suspicious looking cervix must be recorded. The sample taker should ensure that the woman has understood the procedure and is aware of when and how she will receive the test result.

Information should be provided on the request form as to whether the result of the examination has to be sent to another physician (for instance to the general practitioner if the sample is taken by a gynaecologist).

1.5 Transport to the laboratory

After fixation, the conventional slide should be allowed to dry completely. It should then be placed in a cardboard or plastic container for transport to the laboratory. If it is put in the container too quickly, a wet specimen can stick at the edges. The container must be labelled with identification details matching those on the request form.

LBC samples must be placed in a sealed plastic bag with the request form in a separate compartment of the bag as for other clinical samples.

There may be local and manufacturers' regulations about how specimens of human material should be transported, which should be followed.

1.6 Feedback on the quality of the specimen

The cytological report, should use a standard reporting system compatible with the Bethesda System and must include a judgement of the quality of the specimen preferably including information about TZ sampling (see Annex 2; Solomon *et al.*, 2002; Solomon & Nayar, 2004; Herbert *et al.*, 2007). Moreover, if the sample is unsatisfactory, the reasons should be indicated (Solomon & Nayar, 2004).

Every practitioner taking samples for cytology should be provided with periodical summary reports of the quality of their samples in terms of detection of cytological abnormality, specimen adequacy and, preferably also TZ sampling. The reports should be compared with those of other practitioners using the same cytology service. This feedback, provided by the laboratory or a central register, is helpful in improving the average quality of cytological preparations.

1.7 References

- Arbyn M. & Flemish Working Party Sampling. A technical guideline: collection of adequate Pap smears of the uterine cervix. Scientific Institute of Public Health 2000; IPH/EPI-REPORTS 4, 1-53. Available from: http://www.iph.fgov.be/epidemo/epinl/cervixnl/s_eng1.pdf
- Boon M.E. & Suurmeijer A.J.H. (1993). The Pap Smear, second edition edn. Coulomb Press Leyden.
- Bos A.B., van Ballegooijen M., van den Akker van Marle M.E., Hanselaar A.G., van Oortmarssen G.J., & Habbema J.D. (2001). Endocervical status is not predictive of the incidence of cervical cancer in the years after negative smears. *Am. J. Clin. Pathol.* **115**: 851-855.
- BSCC. How to take a cervical smear, 3rd edition (2003). Uxbridge, British Society of Clinical Cytology. Video and booklet available from www.clinicalcytology.co.uk
- Buntinx F. & Brouwers M. (1996). Relation between sampling device and detection of abnormality in cervical smears: a meta-analysis of randomised and quasi- randomised studies. *BMJ* **313**: 1285-1290.
- Buntinx F., Knottnerus J.A., Crebolder H., Essed G., & Schouten H. (1992). Relation between quality of cervical smears and probability of abnormal results. *BMJ* **304**: 1224.
- Burghardt E. (1970). Latest aspects of precancerous lesions in squamous and columnar epithelium of the cervix. *Int. J. Gynecol. Obstet.* **8**: 573-580.
- Burghardt E., Pickel H., & Girardi F. (1998). Colposcopy Cervical Pathology, 3rd revised and enlarged edition. Stuttgart, New York: Georg Thieme Verlag: 1-323.
- Herbert A., Bergeron C., Wiener H., Schenck U., Klinkhamer P.J., Bulten J., & Arbyn M. (2007). European guidelines for quality assurance in cervical cancer screening: recommendations for cervical cytology terminology. *Cytopathology* **18**: 213-219.
- Martin-Hirsch P., Lilford R., Jarvis G., & Kitchener H.C. (1999). Efficacy of cervical-smear collection devices: a systematic review and meta-analysis. *Lancet* **354**: 1763-1770.
- Mitchell H.S. (2001). Longitudinal Analysis of Histologic High-Grade Disease after Negative Cervical Cytology According to Endocervical Status. *Cancer* **93**: 237-240.
- NCCLS (1994). Papanicolaou technique; approved guideline. Pennsylvania: National Comity for Clinical Laboratory Standards, NCCLS Document GP15-A, vol **14** N 8 (video).
- NHSCSP (2006). Taking Samples for Cervical Screening a Resource Pack for Trainers. Sheffield: National Health Service Cervical Screening Programme, NHSCSP Publication **N°23**: 1-47. Available from www.cancerscreening.nhs.uk
- Siebers A.G., de Leeuw H., Verbeek A.L., & Hanselaar A.G. (2003). Prevalence of squamous abnormalities in women with a recent smear without endocervical cells is lower as compared to women with smears with endocervical cells. *Cytopathology* **14**: 58-65.
- Solomon D., Davey D., Kurman R., Moriarty A., O'Connor D., Prey M., Raab S., Sherman M.E., Wilbur D., Wright T.C., & Young N. (2002). The 2001 Bethesda System: terminology for reporting results of cervical cytology. *JAMA* **287**: 2114-2119.

Collection of cellular material of the uterine cervix
Preparation of an adequate Pap smear – ANNEX 1

Solomon D. & Nayar R. (2004). The Bethesda system for reporting cervical cytology: definitions, criteria and explanatory notes. 2nd edition. New York: Springer, 1-191.

Vooijs P.G., Elias A., van der Graaf Y., & Veling S. (1985). Relationship between the diagnosis of epithelial abnormalities and the composition of cervical smears. *Acta Cytol.* **29**: 323-328.

Annex 2

Recommendations for cervical cytology terminology

2.1 Introduction

Cytology reports may include a text report but this should be concise. All reports should include a classification broadly corresponding to the categories described below (and also shown diagrammatically in Fig. 1. The Bethesda system (TBS) was first proposed in 1988 as a model for the interpretation of cervical cell cytology (Lundberg, 1989). The aim was to unify the terminology (Table 1) and thereby improve patient management. Following several years of testing, the system was evaluated in 1991 during a second workshop (Luff, 1992) and was modified again after an inter-national consensus conference in 2001, which forms the basis for the system currently in use throughout much of the world and is summarized in Table 2 (Solomon & Nayar, 2003). The following European guidelines strongly recommend that all terminology systems should be translatable into the categories used by TBS.

2.2 Specimen adequacy

It is inevitable that some cytological specimens will be unsatisfactory for evaluation either because there are too few cells or the cells are poorly fixed or obscured by blood or exudate. The assessment of adequacy is subjective and the cytologist should provide in the text report their reason for that assessment (see also Chapter 3, Section 3.5).

Laboratories are recommended to use TBS criteria for adequacy as a minimum, requiring at least 8,000 – 12,000 squamous cells on a conventional smear and at least 5,000 cells on a liquid-based preparation. Comments may be given on the report about inflammatory exudate and transformation zone sampling on conventional smears and liquid-based preparations so that nurses and doctors taking the samples may make clinical decisions as to whether the test should be repeated. In the UK, tests are not repeated unless recommended by the laboratory, and local protocols may be more stringent than those recommended by TBS (NHSCSP, 2000).

The European guidelines and TBS state that a judgement on sample quality must be given as to whether the sample is regarded as satisfactory or not (Solomon & Nayar, 2003; see Chapter 4 section 4.5.2. and 4.6.1). Evidence of transformation zone sampling should be recorded although this is not a requirement on its own for a satisfactory sample (Solomon & Nayar, 2003; NHSCSP, 2000).

2.3 General categorization

This is an optional category in TBS, which allows for statistical analysis of principal categories: negative for epithelial lesion or malignancy, epithelial abnormalities, and other (see Table 2).

2.4 Interpretation/result

2.4.1 Negative for intraepithelial lesion or malignancy

The category "negative for intraepithelial lesion or malignancy" in TBS regroups the categories "normal and benign alterations".

Numerous variants of benign cellular findings have been described and need not be reported if they do not imply an increased risk of neoplasia: these include hormonal patterns (post-partum or atrophic), repair changes, microglandular hyperplasia, tubo-endometrioid metaplasia, tubal metaplasia, sampling of the lower uterine segment, irradiation changes or alterations due to inflammation or the presence of an intrauterine contraceptive device (IUD) and benign glandular cells occasionally seen in post-hysterectomy specimens (Tambouret *et al.*, 1998). As long as these changes are recognised as such they need not be reported. The presence of certain organisms, such as *Trichomonas vaginalis*, *Candida*, *Actinomyces*-like organisms and Herpes virus multinucleated cells may be reported as they have potential clinical relevance.

2.4.2 Cells indicating a squamous intraepithelial lesion/neoplasia/dysplasia

There are many linguistic and terminological differences in the systems used to describe the spectrum of precancerous cell change, still widely described as mild, moderate and severe dysplasia/carcinoma in-situ (Riotton *et al.*, 1973), which broadly correlates to cervical intraepithelial neoplasia (CIN) grades 1-3 (Richart, 1973). In cytology, the dysplasia / CIN spectrum has been simplified in TBS as low-grade and high-grade squamous intraepithelial lesion (LSIL and HSIL) (Solomon & Nayar, 2003; Solomon *et al.*, 2002). The NHSCSP continues to use the descriptive term "dyskaryosis" for cytology, which broadly correlates to CIN on histology (NHSCSP, 2000) but the British Society for Clinical Cytology (BSCC) has proposed to move to a two-tier system of low-grade and high-grade dyskaryosis equivalent to LSIL and HSIL (Herbert, 2004; Herbert, 2005). Numerical systems (Papanicolaou I-V) should not be used: textual systems such as dyskaryosis, and SIL are nowadays recommended in preference. CIN should be used for histology rather than cytology.

It is sometimes difficult for countries to change their terminology (and there will always be linguistic differences) but it is strongly recommended that all local cytology terminologies should be translatable into the TBS since the latter is used so widely in the world today. The old WHO classification recognises three grades of dysplasia (mild, moderate and severe) and carcinoma in situ. For all practical purposes severe dysplasia may be merged with carcinoma in situ. In TBS, which is now used by WHO, LSIL equates to HPV/mild dysplasia / CIN1 and HSIL to moderate and severe dysplasia, carcinoma in situ / CIN2 and CIN3 (Table 2) (WHO, 2003).

2.4.2.1 LSIL, mild dysplasia, cellular changes suggesting CIN1

LSIL includes changes known to be associated with infection by human papillomavirus (HPV), most obviously manifest by koilocytosis. LSIL cannot be distinguished from transient HPV infection by

cytology alone, which is the rationale for surveillance to identify the minority that progress to high-grade lesions. LSIL in TBS and mild dyskaryosis in NHSCSP correspond to the histopathological diagnoses mild dysplasia, and CIN1 (NHSCSP, 2000).

2.4.2.2 HSIL, cellular changes suggesting CIN2 / moderate dysplasia

CIN2 is an intermediate grade, in which the changes fall short of CIN3 / carcinoma in-situ. CIN2 is equivalent to moderate dysplasia and moderate dyskaryosis and is included in HSIL. Cytological reports of HSIL or high-grade dyskaryosis may include a text report favouring CIN2 or moderate dyskaryosis. Most terminological systems already link moderate with severe dysplasia as high-grade lesions and this is strongly recommended. Whether or not clinical management of moderate dysplasia is different from severe dysplasia, moderate dysplasia should be classified as high-grade rather than low-grade.

Some systems (such as the Munich system) link moderate with mild dysplasia, which is the only significant difference among European terminologies. A European panel discussion on this subject reported in *Cytopathology* came to the conclusion that those systems "linking moderate dysplasia with mild rather than severe dysplasia would need to define moderate dysplasia as such, if their results were to be translatable, which would be preferable to their using a different definition of low-grade and high-grade lesions" (Kocjan *et al.*, 2005).

2.4.2.3 HSIL, cellular changes suggesting CIN3 / severe dysplasia / carcinoma in situ

HSIL, suggesting CIN3, is the cytological equivalent of severe dysplasia and carcinoma in-situ. HSIL includes moderate and severe dyskaryosis but the text report may favour CIN3 or severe dyskaryosis.

2.4.2.4 Invasive squamous cell carcinoma

The diagnosis of invasive cancer requires a histological biopsy but there are cytological changes that suggest the possibility of invasion. Most systems, including TBS, recognise the importance of reporting such changes and define a separate category for the commonest type of invasive cancer (squamous cell carcinoma) or for changes in which the cell type of invasive cancer is not evident.

2.4.2.5 Atypical / borderline squamous cells

In practice, with all terminologies, atypical / borderline changes are frequently reported although the category should be reserved for cases in which there is genuine doubt as to whether the changes are reactive or neoplastic. Most of these changes border on LSIL / mild dysplasia (Quality Assurance Reference Centre for the Trent NHSCSP, 2002) and are described in TBS as atypical squamous cells of undetermined significance (ASC-US). It has been decided to keep this category, which has been shown to be associated with approximately 10% of CIN2-3 on biopsies (Davey *et al.*, 2000; Arbyn *et al.*, 2004). Not more than 3% of the smears should have this designation (Davey *et al.*, 2000) but rates will depend on local rates for LSIL and HSIL. When recognised as such, reactive changes associated with inflammation come out of this group and should now be included among normal smears. These recommendations are similar to those for "borderline, not otherwise specified" in the proposed BSCC classification (Herbert, 2004).

2.4.2.6 Atypical squamous cells – high-grade not excluded (ASC-H)

ASC-H is a sub-group of atypical / borderline changes in which the changes are suspicious of HSIL and occasionally cancer. It is sometimes used when the abnormal cells are so few that the diagnosis is uncertain. Most systems recommend that these cases, which should be unusual, should be identified in text reports or as a separate category. The BSCC proposes to call this category "borderline, high-grade not excluded" (Herbert, 2004). This term should apply to no more than 5-10% of atypical squamous cell alterations and are often associated with CIN2-3 confirmed on colposcopically directed biopsy (Sherman *et al.*, 1999; Quddus *et al.*, 2001; Sherman, Solomon & Schiffman, 2001). The use of this term should be monitored and controlled in order to avoid its use for recognizable HSIL / high-grade dyskaryosis.

2.4.3 Glandular cell abnormalities

Glandular lesions are less common than their squamous cell counterparts but form an important group as they are more difficult to detect by cytological screening and more difficult to recognise at colposcopy.

2.4.3.1 Endocervical adenocarcinoma in situ

AIS is defined as a recognisable sub-type in many terminologies including TBS. It corresponds to high-grade CGIN but as there are no clear criteria for diagnosing low-grade CGIN on cytology, CGIN is usually reported and managed as one entity.

2.4.3.2 Adenocarcinoma

As with squamous cell carcinoma, the diagnosis of invasion requires a histological biopsy. In some instances there are cytological changes suggesting invasive adenocarcinoma. In the UK, the difficulty of distinguishing in situ from invasive adenocarcinoma is recognised and these entities are included as "glandular neoplasia" (NHSCSP, 2000). It may be possible to distinguish cytological changes suggesting endometrial or extra-uterine from endocervical adenocarcinoma and this should be made clear in the text report.

2.4.3.3 Atypical / borderline changes in glandular cells

As with squamous cell changes, there are some instances when equivocal glandular cell changes are reported on cytology although the relative rarity of glandular neoplasia should make this unusual. TBS identifies a separate group of "atypical glandular cells" and also the BSCC proposes to separate "borderline changes in glandular cells" from the far commoner borderline changes in squamous cells. Glandular cell changes in cervical cytology are diverse and, where possible, text reports should distinguish changes likely to be endometrial rather than endocervical. Occasionally, such as in the presence of an IUD, atypical / borderline changes in glandular cells may be considered likely to be benign and an early repeat may be recommended for re-assurance. Such changes should be investigated if they persist on a second occasion. Furthermore, if the changes on any occasion are thought to favour glandular neoplasia, but are insufficient for a firm diagnosis, the category "atypical glandular cells suggesting neoplasia" has been proposed by TBS. This category is badly defined on morphological grounds (Lee *et al.*, 1991; Biscotti *et al.*, 1997; Soofer & Sidawy, 2000)

but since the observation of atypical glandular cells is often associated with underlying neoplasia or cancer, a recommendation for investigation is warranted.

2.4.4 Other Cellular changes

Cervical cytology is not a good diagnostic assay for endometrial cancer. Morphologically benign endometrial cells were not mentioned in the 1988 Bethesda system, except referring to menopausal women. The category "other" is now proposed to classify smears without morphological abnormalities but which have apparently benign endometrial cells, in women over 40 years. The presence of these cells indicates an increased risk for endometrial cancer, and therefore requires endometrial exploration (Montz, 2001; Fadare *et al.*, 2005). Benign glandular cells may be found after total hysterectomy and need not be reported (Tambouret *et al.*, 1998).

2.5 Additional remarks

2.5.1 Automated review

The automated system for reading slides should be mentioned in the report and the printout from the machine attached. If the slide was rechecked by microscopy, this should also be mentioned separately in the report.

2.5.2 Ancillary testing

It is considered useful to propose recommendations for additional tests, which may be complementary to cytology. High-risk HPV DNA detection is a prime example of an additional test that can be complementary to cytology for a diagnosis of ASC-US.

2.5.3 Educational notes and suggestions

Recommendations for patient management should be clear and concise. They must be given as "suggestions" and in accordance with national and international good clinical practice.

2.6 Summary

If the principles of this classification are used, there should be more similarities than differences between terminologies used across Europe and it should be possible for any system to be translatable into TBS. Throughout these guidelines, the CIN classification is reserved to describe histological lesions, whereas TBS is used for cytological abnormalities.

No European equivalent of TBS can be proposed as the only unique classification system for the EU but all systems should at least be translatable into TBS. Cytological classification systems may continue to use three-tier systems within the framework of TBS. Nevertheless, each member state should define a nationally agreed reporting scheme. A three-tier system distinguishing 1) mild dysplasia or dyskaryosis (including HPV associated lesions), 2) moderate dysplasia or dyskaryosis and 3) severe dysplasia or dyskaryosis is perfectly acceptable as long as moderate and severe are linked as high-grade. A two-tier system lumping mild and moderate dysplasia into one category is not recommended. The fact that, in certain countries, women with a first result of moderate dysplasia are followed-up conservatively is not a sufficient reason to link mild with moderate.

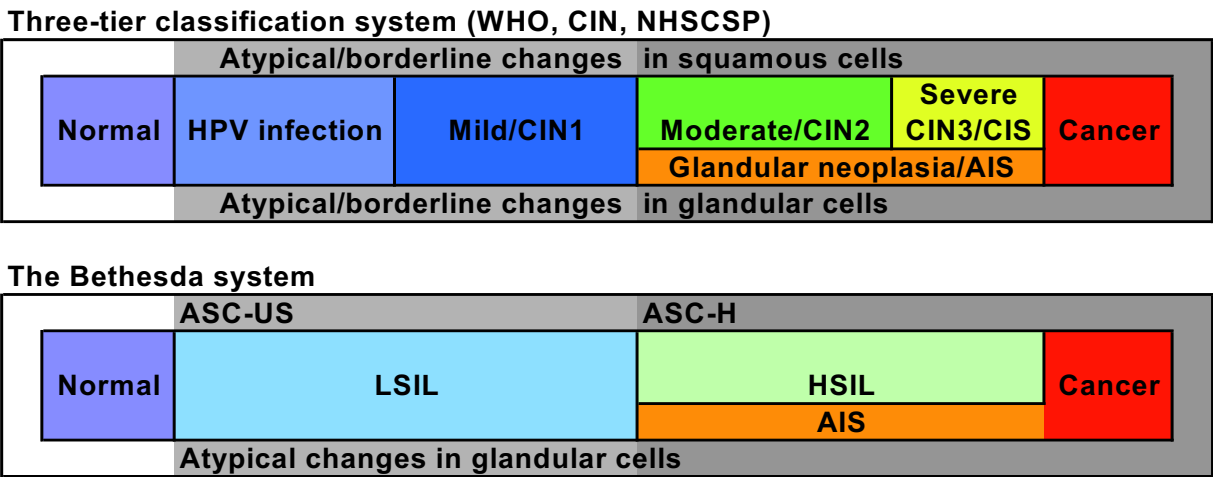


Fig. 1. Conceptual categorisation of cytological findings in a Pap smear of the uterine cervix¹

¹ Tables reprinted from Herbert A. (2004). *BSCC terminology for cervical cytology: two or three tiers? why not five, seven or even 14? Cytopathology*; 15: 245-251 with permission from Blackwell Publishing Ltd. and from Herbert A. (2005). *Terms without borders: An international approach to terminology. Diagn.Cytopathol*; 33: 352-355. with permission from John Wiley & Sons, Inc.

Table 1. Conversion table for different cytological classification systems

Papanicolaou	WHO		CIN (Richart, 1973)	TBS 1991 (Luff, 1992)	TBS 2001 (Solomon & Nayar, 2003)
I	Normal				Negative for epithelial abnormality
II	Atypia			Infection, reactive repair	
				ASCUS	ASC-US
	Atypical glandular cells			AGUS	ASC-H
III	Atypical glandular cells			AGUS	Atypical glandular cells
	Mild dysplasia	Condyloma	LSIL	LSIL	LSIL
		CIN I			
IV	Moderate dysplasia	CIN II	HSIL	HSIL	HSIL
	Severe dysplasia	CIN III			
	CIS				
	AIS	CGIN	AGUS		AIS
V	Invasive carcinoma				

Table 2. The 2001 Bethesda system: terminology for reporting the results of cervical cytology²

SPECIMEN ADEQUACY

1. Satisfactory for evaluation (note presence/absence of endocervical/ transformation zone component)
2. Unsatisfactory for evaluation . . . (specify reason)
 - Specimen rejected/not processed (specify reason)
 - Specimen processed and examined, but unsatisfactory for evaluation of epithelial abnormality because of (specify reason)

GENERAL CATEGORIZATION (Optional)

1. Negative for intraepithelial lesion or malignancy
2. Epithelial cell abnormality
3. Other

INTERPRETATION/RESULT

1. Negative for Intraepithelial Lesion or Malignancy
 - Organisms
 - Trichomonas vaginalis
 - Fungal organisms morphologically consistent with Candida species
 - Shift in flora suggestive of bacterial vaginosis
 - Bacteria morphologically consistent with Actinomyces species
 - Cellular changes consistent with herpes simplex virus
 - Other non-neoplastic findings (Optional to report; list not comprehensive)
 - Reactive cellular changes associated with inflammation (includes typical repair)
 - Radiation
 - Intrauterine contraceptive device
 - Glandular cells status posthysterectomy
 - Atrophy
2. Epithelial Cell Abnormalities
 - Squamous cell
 - Atypical squamous cells (ASC) of undetermined significance (ASC-US)
 - Atypical squamous cells cannot exclude HSIL (ASC-H)
 - Low-grade squamous intraepithelial lesion (LSIL), encompassing: human papillomavirus/mild dysplasia/cervical intraepithelial neoplasia (CIN) 1
 - High-grade squamous intraepithelial lesion (HSIL), encompassing: moderate and severe dysplasia, carcinoma in situ; CIN 2 and CIN 3
 - Squamous cell carcinoma
 - Glandular cell
 - Atypical glandular cells (AGC) (specify endocervical, endometrial, or not otherwise specified)
 - Atypical glandular cells, favor neoplastic (specify endocervical or not otherwise specified)
 - Endocervical adenocarcinoma in situ (AIS)
 - Adenocarcinoma
3. Other (List not comprehensive)
 - Endometrial cells in a woman 40 years of age

AUTOMATED REVIEW AND ANCILLARY TESTING (Include as appropriate)

EDUCATIONAL NOTES AND SUGGESTIONS (optional)

² Table reprinted from Solomon D., Davey D., Kurman R. et al. (2002). *The 2001 Bethesda System: terminology for reporting results of cervical cytology*. JAMA; **287**: 2114-2119 with permission from American Medical Association © 2002 All Rights reserved.

2.7 References

- Arbyn M., Buntinx F., Van Ranst M., Paraskevaidis E., Martin-Hirsch P., Dillner J. (2004). Virologic versus cytologic triage of women with equivocal Pap smears: a meta-analysis of the accuracy to detect high-grade intraepithelial neoplasia. *J. Natl. Cancer Inst.*; **96**: 280-293.
- Biscotti C.V., Gero M.A., Toddy S.M., Fischler D.F., Easley K.A. (1997). Endocervical adenocarcinoma *in situ*: an analysis of cellular features. *Diagn. Cytopathol.*; **17**: 326-332.
- Davey D.D., Woodhouse S., Styer P.E., Stastny J., Mody D. (2000). Atypical epithelial cells and specimen adequacy: current laboratory practices of participants in the College of American Pathologists Interlaboratory Comparison Program in Cervicovaginal Cytology. *Arch. Pathol. Lab. Med.*; **124**: 203-211.
- European Commission (in press). European Guidelines for Quality Assurance in Cervical Cancer Screening. Eds: Arbyn M., Anttila A., Jordan J., Ronco G., Schenck U., Segnan N., Weiner H. Luxembourg: Office of Official Publ. EU.
- Fadare O., Ghofrani M., Chacho M.S., Parkash V. (2005). The significance of benign endometrial cells in cervicovaginal smears. *Adv. Anat. Pathol.*; **12**: 274-87.
- Herbert A. (2004). BSCC terminology for cervical cytology: two or three tiers? Why not five, seven or even 14? *Cytopathology*, **15**: 245-251.
- Herbert A. (2005). Terms without borders: An international approach to terminology. *Diagn. Cytopathol.*; **33**: 352-355.
- Kocjan G., Priollet B.C., Desai M. *et al.* (2005). BSCC, Bethesda or other? Terminology in cervical cytology European panel discussion. *Cytopathology*, **16**: 113-119.
- Lee K.R., Manna E.A., Jones M.A. (1991). Comparative cytologic features of adenocarcinoma *in situ* of the uterine cervix. *Acta Cytol.*; **35**: 117-126.
- Luff R.D. (Chairman) (1992). The revised Bethesda system for reporting cervical/vaginal cytological diagnoses. Report of the 1991 Bethesda Workshop. *Acta Cytol.*; **36**: 273-276.
- Lundberg G.D., National Cancer Institute (1989). The 1988 Bethesda System for Reporting Cervical/Vaginal Cytologic Diagnoses. *JAMA*; **262**: 931-934.
- Montz F.J. (2001). Significance of "normal" endometrial cells in cervical cytology from asymptomatic postmenopausal women receiving hormone replacement therapy. *Gynecol. Oncol.*; **81**: 33-39.
- National Health Service Cervical Screening Programme (2000). Achievable standards, benchmarks for reporting, and criteria for evaluating cervical cytopathology. Second edition including revised performance indicators (2nd edition). *NHSCSP publication 1*, 1-36. Sheffield, NHS Cancer Screening Programmes.
- Quality Assurance Reference Centre for the Trent NHSCSP (2002). Do borderline nuclear changes in gynaecological cytology constitute a reliable reporting category? *Cytopathology*, **13**: 220-231.
- Quddus M.R., Sung C.J., Steinhoff M.M., Lauchlan S.C., Singer D.B., Hutchinson M.L. (2001). Atypical squamous metaplastic cells: Reproducibility, outcome, and diagnostic features on ThinPrep Pap test. *Cancer*; **93**: 16-22.
- Richart R.M. (1973). Cervical intraepithelial neoplasia. *Pathol. Annu.*; **8**: 301-323.

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Riotton G., Christopherson W.M., Lunt R. (1973). Cytology of the Female Genital Tract. International Histological Classification of Tumours **No. 8**. *World Health Organisation*, Geneva.

Sherman M.E., Solomon D., Schiffman M.A. (2001). Qualification of ASCUS. A comparison of equivocal LSIL and equivocal HSIL cervical cytology in the ASCUS LSIL Triage study. *Am. J. Clin. Pathol.*; **116**: 386-394.

Sherman M.E., Tabbara S.O., Scott D.R. *et al.* (1999). "ASCUS, rule out HSIL": cytologic features, histologic correlates, and human papillomavirus detection. *Mod. Pathol.*; **12**: 335-342.

Solomon D., Davey D., Kurman R. *et al.* (2002). The 2001 Bethesda System: terminology for reporting results of cervical cytology. *JAMA*; **287**: 2114-2119.

Solomon D., Nayar R. (2004). The Bethesda system for reporting cervical cytology: definitions, criteria and explanatory notes, 2nd edn. *Springer*, New York.

Soofer S.B., Sidawy M.K. (2000). Atypical glandular cells of undetermined significance: clinically significant lesions and means of patient follow-up. *Cancer*; **90**: 207-214.

Tambouret R., Pitman M., Bell D.A. (1998). Benign glandular cells in posthysterectomy vaginal smears. *Acta Cytol.*; **42**:1403-8.

Tavassoli F.A., Devilee P. (Eds) (2003). World Health Organization Classification of Tumours. Pathology and genetics of tumours of the breast and female genital organs. *IARC Press*. Lyon.

4

Laboratory guidelines and quality assurance practices for cytology

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4.1 Executive summary

The quality of a cervical cytology laboratory depends on adequate handling and staining of the samples, screening and interpretation of the slides and reporting of the results. This chapter gives an overview of procedures recommended in Europe to manage the balance between best patient care possible, laboratory quality assurance and cost effectiveness.

4.2 Introduction

The microscopic examination and interpretation of histological and cytological specimens is a subjective procedure, highly dependent on the skills and experience of the investigator and the time spent on examination of the cell/sample (Klinkhamer *et al.*, 1988; Koss, 1989; O'Sullivan *et al.*, 1996). Inter- and intra-observer variation and the high variance in percentages of correct diagnoses described in the literature are a logical consequence (Grenko *et al.*, 2000; Stoler & Schiffman, 2001; Stoler, 2002).

The aim of optimal quality assurance is to provide the best possible patient care. With respect to cervical screening this means a balance between manageable control of costs and low false test result rates. Beyond correct sampling of the cervix, the quality of the test depends on the subsequent steps: adequate handling and staining of the sample, screening and interpretation of the slide and reporting of the results as well as the final step of assuring accuracy.

4.3 Personnel and organisation

4.3.1 General

The laboratory should be staffed by well trained personnel headed by a medical professional. The cytology laboratory (or group of collaborating cytology laboratories) should process a sufficient number of tests to be able to maintain adequate expertise. There is insufficient data to make a definitive evidence-based statement about the number of smears necessary for this purpose, but it is the professional opinion of the chapter authors that at least 15,000 tests per year should be processed in a laboratory participating in organised screening.

The position of each employee in the pathology laboratory should be recorded in an organisational document to allow performance at all levels to be monitored.

4.3.2 Requirements for cytotechnologists

4.3.2.1 Cytotechnologist

In cervical cancer screening the main task of cytotechnologists is the primary screening of cervical smears of women without specific symptoms. To reach the goal of correctly identifying precursor lesions, administrative tasks, technical laboratory tasks, monitoring of follow-up results and activities related to quality assurance, and archiving slides and results are included in the working process of cytotechnologists. Their work is done under supervision, as will be described in sub-sequent chapters.

- Administrative tasks include contact with patients, smear takers, general practitioners (GPs), gynaecologists, other laboratories and hospitals. Cytotechnologists must respect patient confidentiality and must be trained in country-specific legal requirements.
- Technical laboratory tasks include handling specimens, carrying out relevant laboratory techniques and performing prescribed health and safety procedures.
- Participation in continuing education, feedback sessions (Tarkkanen *et al.*, 2003), and quality control programs is mandatory for all cytotechnologists.

Principles and practices should be learned prior to taking part in the routine work of the laboratory. The educational basis for (licensed) cytotechnologists differs within the European countries (see Table 1 for examples).

4.3.2.2 Senior cytotechnologist

The senior cytotechnologist will usually be responsible for internal quality control of all steps within the screening process, including administration, staining and microscopic cytodiagnosis, and should be familiar with external quality protocols. A minimum of 5 years' experience in gynaecological cytology is usually required.

Specific tasks of the senior cytotechnologists may be:

- Daily management of the cytopathology laboratory, including personnel affairs and staff appraisal.
- Direction of laboratory technicians in sample preparation.
- Assistance and supervision of lower-level cytotechnologists in the performance of analytical procedures and tests.
- Communication with the cytopathologist to whom they are responsible.
- Management of periodical circulation and discussion of special cases among cytotechnologists, and between cytotechnologists and cytopathologists.
- Timely forwarding of cytology reports to the regional or national cancer screening registry according to current directives.
- Assistance in the maintenance of supplies, equipment, and instruments, and in the day-to-day function of the laboratory.
- Assistance of scientists in the same program area.

Step-wise screening and review of slides with abnormalities initially identified by cytotechnologists may be done by the senior cytotechnologist responsible for the management of the laboratory or by other cytotechnologists with similar experience and training in gynaecological cytology. In the United Kingdom senior cytotechnologists may take a Certificate in Advanced Practice in Cervical Cytology, which qualifies them to report cervical cytology slides, including signing out abnormal cases, under the overall responsibility of the cytopathologist leading the laboratory (Smith & Hewer, 2003).

Table 1. Examples of minimal educational requirements for screeners working in gynaecological cytology

Country	General education required	Specific education/training required		Exams/certificates required
Austria	Grammar school	Academy for medico-technical laboratory service, 3 years including a 40-60 hours course in cytology , with exam	Training within the laboratory without time limit, local courses	Leaving certificate of the Academy for medico-technical laboratory service
Belgium (Drijkoningen <i>et al.</i> , 2005)	Not specified	No schools for long-term training	Training within the laboratory without time limit	Certificate not obligatory
Bulgaria (Valkov <i>et al.</i> , 2004)	Grammar school	Study of biology		Not specified
Czech Republic (Bekova & Kobilkova, 2005)	Not specified	School for gynaecological cytology 1 year		Exam in gynaecological cytology
Denmark	Not specified	Laboratory school, 3.5 years	Educational program within the hospital (specific for each region), local courses	QUATE-exam, voluntary
France	Not specified	Private school for cytology, 1 year	Specific training within the laboratory	Certificate not obligatory
Germany (Schenck <i>et al.</i> , 1998)	Not specified	School for cytology, 2 years	Additional training within a certified laboratory for at least one year	Voluntary certificate
United Kingdom	General certificate of education (4 o-level equivalent)	2 year training, includes NHSCSP course	In-house training, logbook, min 5,000 slides screened.	NHSCSP Certificate of Competence in Cervical Cytology
Italy	Grammar school	College of health care profession including a course in cytology	Specific training within the laboratory	Thesis (laurea preve)
The Netherlands	Grammar school	Laboratory school, 4 years (medium or high-level)	Specific training within a certified laboratory for at least one year	Certificate not obligatory

4.3.3 Requirements for other technical laboratory personnel

Technical laboratory personnel must be educated and experienced in accordance with their role. Technical personnel must be able to:

- handle relevant laboratory techniques according to guidelines and procedure descriptions;
- perform prescribed health and safety procedures; and
- take part in specific quality control programs.

4.3.4 Requirements for a cytopathologist

The cytopathologist is responsible for the final assessment of cervical samples. Specific tasks of the cytopathologist with respect to cervical cytology are:

- Assessment and authorisation of all cases referred to the clinician for further follow-up or treatment.
- Resolving discrepancies between the diagnoses of cytotechnologists, if those diagnoses would lead to differing recommendations to the requesting physician.
- Review and intra-laboratory discussion of cases showing serious discrepancy between the cytological and/or histological follow-up (Tarkkanen *et al.*, 2003)
- Communication with gynaecologists and other sample takers with respect to specific cases. Communication includes a periodical report to smear takers with respect to the quality aspects of the samples.
- Communication and education of cytotechnologists with respect to difficult cases and cases with discrepant cyto-histological results.
- Guidance and support for adequate (continuing) education of cytotechnologists and junior medical staff.
- Participation in quality assurance programs including preparation of an annual report concerning the outcomes of the cytological and histological follow-up examinations (e.g. Breitenacker *et al.*, 2004).

4.3.5 Requirements for administrative personnel

Secretarial and administrative employees:

- should be educated in relevant medical terminology;
- should be able to work with current word processors and with automated database systems; and
- must respect patient confidentiality.

4.3.6 Final responsibility

Final responsibility is dependent on national legal regulations. In general, medical specialists additionally certified for cytopathology are responsible for the management of the laboratory.

4.4 Material requirements

4.4.1 Buildings, rooms and furniture

Buildings, rooms and furniture must comply with regional and federal legal requirements. Proper working conditions require that:

- the laboratory is located, constructed and equipped in such way that all functions can be properly performed within agreed safety standards. All areas should be well lit, well ventilated, quiet and spacious;
- the screening room, the sample-preparation room and the secretarial room should be separate rooms;
- the specimen preparation area must be equipped with effective exhaust systems and approved biohazard hoods, together with adequate counter space and sinks;
- there must be adequate storage containers for flammable and poisonous chemicals;
- cytotechnologists should have a comfortable chair with adequate back support and ample desk space to permit microscopic examination and record keeping; and
- adequate measures should be taken to prevent repetitive motion injuries and other injuries due to ergonomic problems.

Guidelines for procedures in case of emergencies must be known by all personnel and safety manuals must be easily available.

4.4.2 Equipment for staining, microscopes, record systems and teaching materials

For cervical screening cytology the Papanicolaou stain, original or modified, is recommended (Papanicolaou, 1942; Gill *et al.*, 1974).

- The equipment needed depends on whether staining is automated or manual. After staining, cytological material should present well-stained chromatin, differential cytoplasmic counter-staining and cytoplasmic transparency (Koss, 1992).

A high-quality binocular microscope should be available for all screening staff and should be regularly serviced, including a check of its technical set up that includes adequacy of the stage and objectives.

- For conventional cytology 4x, 10x and 40x objectives are essential. 4/5x objectives should be present to allow convenient marking of the cells of interest (Koss, 1992).
- For liquid-based cytology (LBC) 20x objectives are required.

Screening personnel should enter their cytological results onto a computerized system to allow quality assessment.

Relevant textbooks and journals should be easily available and accessible.

4.5 Handling and analysis of cervical samples

4.5.1 Laboratory preparation

All laboratory procedures should be registered and allocated to an appropriate member of the staff. All personnel should be familiar with safety guidelines and procedures in case of emergency.

- When delivered, all specimens (slides or vials) should be accompanied by a request form giving as a minimum the patient's identification data, data of the physician in charge and clinical information including the appearance of the cervix, method of contraception and stage of menstrual cycle.
- Any irregularities concerning the clinical data sheet and/or the cytological specimen should be recorded and resolved if possible in communication with the person sending the test.
- After verification of correct correlation of the sample and the corresponding request form both should be labelled with a unique identification number.
- Prior to the assessment of the sample, the patient's screening history should be retrieved from the local laboratory files and/or screening data base and be made available to the cytotechnologist.
- Spray-fixed smears should be soaked in ethanol or water before the staining procedure.
- Liquid-based specimens should be processed according to the manufacturer's instructions.
- The slides should be stained according to a standard Papanicolaou protocol (including control of staining).
- The samples should have a cover-slip that covers all the cellular material (usually 50 by 24 mm), and labelling should be checked before the slide is screened.

4.5.2 Assessment of the sample: stepwise screening

4.5.2.1 Initial assessment

Primary screening is performed by cytotechnologists.

- Slides should be placed in the mechanical stage holder of the microscope with the label always on the same side (Koss, 1992).
- In conventional slides, the cover-slipped area should be screened completely, in horizontal or vertical directions using overlapping screening-patterns. In liquid-based specimens the entire area within the circle should be screened. Microscope process control systems equipped with electronic marking capability may be helpful in quality assessment (Schenck & Planding, 1996).
- Unusual and/or abnormal cells should be marked (manually or computer-guided).
- Repeat samples should be compared with the sample on which the recommendation was given.
- The results should be reported according to a national standard classification system. A statement about the quality of the cervical sample should be included. In case of unsatisfactory samples, a repeat test should be advised.
- Conclusion and recommendations, including those for repeat smears and referral for gynaecological, colposcopic or histologic examinations should be given in concordance with guidelines (see Chapters 5 & 6).
- Reports must show the identity of the cytotechnologist/cytopathologist responsible for the conclusion and recommendation.

4.5.2.2 Samples qualifying for a second screening assessment

The following cases should be re-screened by a second person:

- Samples with inadequate/unsatisfactory quality.
- Samples with any cellular abnormalities leading to a specific recommendation.
- Samples with previous recommendations for a repeat or reference for gynaecological, colposcopic and histological examinations.
- Other high-risk samples according to clinical information or patient history, including:
 - first normal cytology after abnormal cytology or histology,
 - samples of clinical suspect cases (abnormal discharge, postmenopausal bleeding, abnormal or suspicious cervix),
 - negative samples prior to a sample classified as abnormal and initiating further clinical treatment (maximum five years),
 - samples of postmenopausal women with atrophic, difficult to classify, probably abnormal cells with an advice for a repeat sample after short term oestrogen treatment.
- Quality control related slides.

According to national regulations these procedures may be done either by one cytotechnologist and/or one cytopathologist or two cytotechnologists (e.g. The Netherlands, United Kingdom).

4.5.3 Workload requirements – primary screening

A reasonable maximum workload in terms of number of slides per day to be screened should be established within the laboratory and should depend on the method of sample preparation (conventional cytology or liquid-based). Additional work done by the cytotechnologist including staining, quality control procedures and other activities should be taken in account. Within Europe maximum official workload limits are given for slides to be screened by cytotechnologists per day and vary between 25 and 80 cases (Mody, 2000). Some countries give a maximum workload per hour, e.g. in Germany (maximum 10 cases per hour).

It is advised:

- that continuous screening not exceed 2 hours without a break, and
- that primary screening does not exceed six hours per day.

A record of primary screening assessments of individual cytotechnologists and the final signed results should be kept and be retrievable for quality control purposes.

4.5.4 Archiving

The laboratory staff are responsible for proper administration and archiving of request forms, samples and written and/or computerized reports. Procedures must comply with national legislation, including that relating to patients' data security.

Request form: The request forms or their electronic equivalent should be stored for a minimum of three months.

Samples: All slides must be stored for a minimum of 10 years in conditions adequate for preservation. This is important for patient management as well as quality control.

Reports: The storage of written or computerized reports is primarily dependent on national regulations. It is recommended that the reports should be stored for a minimum of 10 years. It is a great advantage to keep coded records of cytology results for future reference, even if the results and slides are no longer available.

Archived Pap smears and histological blocks of cervical tissue constitute a very important source for bio-bank research. The European Union is currently promoting systems allowing high-quality research using stored human biological material (<http://www.cancerbiobank.org/>).

4.6 Recording of results

4.6.1 Laboratory information system

There must be an adequate record-keeping system, preferably computerized. It must be accurate and easily accessible to all laboratory personnel.

The record system should include at least:

- patient identification data,
- name and address of the laboratory,
- laboratory ID number,
- date of arrival of the smear in the laboratory,
- indication for examination: screening, follow-up or clinical indication,
- type of examination: cytological, histological or virological,
- the results of the laboratory examination in accordance with the current standard classification system (see below) and data format, including a judgment of the quality/adequacy of the preparation,
- advice for repeat sample or referral,
- date of the final report, and
- name of the person or persons who evaluated the sample.

The European guidelines recommend that cytology results should be reported using a nationally agreed-upon terminology that is at least translatable into the Bethesda system (Solomon et al 2002); see Chapter 3, Annex 2.

Further requirements are that the information system should

- link multiple test results for the same patient,
- provide easy access to details about previous cervical cytology and histology of the patient,
- provide a mechanism for ascertaining and recording clinical outcome after cytology tests, including colposcopy findings, biopsies, reasons for biopsies not being taken, and
- provide the data necessary for evaluation of the population screening program. All or a selection of the recorded data mentioned above must be forwarded to the national or regional cancer screening registry, according to current directives, and be held at the screening centre for its own evaluation.

4.6.2 Authorisation of results

Every report must be checked for inconsistencies before authorisation and may then be manually or electronically authorised.

Depending on national legal requirements, the cytological reports may be signed either by cytotechnicians or cytopathologists in charge.

4.6.3 Laboratory response time

All efforts should be directed to report results of the screening within 10 working days counted from specimen arrival within the laboratory. If the above-mentioned time limit cannot be met, the referring doctor should be informed.

4.7 Quality management

A variety of concepts in quality management (quality assurance) have been developed as active prevention programs. Generic models (total quality management) like the model established by the European Foundation for Quality Management differ from those based on implementation of international norms/standards (Arcelay *et al.*, 1999). A proper quality management program will help to ensure optimal patient care and minimize the risk of liability claims (Mody *et al.*, 2000).

4.7.1 Internal quality management

4.7.1.1 Laboratory quality management (pre-analytical quality management)

The laboratory must designate a person who, in addition to daily work in cervical screening, is trained in collecting and managing documents, process descriptions and manuals, and is either a trained quality manager or is able to communicate with trained quality managers. Handbooks with practical guidance appear helpful (Council of Europe, 2001).

General management documents should include:

- overview of the screening laboratory,
- description of personnel organisation (including levels of competence and responsibilities of each person, lines of communication and infrastructure), and
- structure of management documents.

The process network should include:

- customer definition,
- management processes,
- core processes, and
- processes of improvement and resources.

The detailed process description should include:

- step-wise slide screening protocols,
- description of personnel responsible for specific processes, and
- methods of detecting and minimizing errors (e.g. checklists).

The whole staff must be informed, and the protocols should be checked yearly and adjusted according to continuing medical education of all personnel.

4.7.1.2 Analytical quality management (cytology)

Accuracy of screening must be monitored with previously agreed-upon protocols for defining and dealing with genuine poor performance so that laboratory morale is maintained and expectations are not too high. Measurements of screening accuracy should also account for variations in accuracy of the final report, which must also be monitored. Methods used for quality assessment should increase dialogue within the lab and improve individual screening accuracy.

There are three main methodologies for internal quality control of cytology:

- Methods based on re-screening of slides,
- Methods based on monitoring screening detection and reporting rates, and
- Methods based on correlation of cytology with clinical/histological outcome.

Internal quality control based on re-screening of slides

Multiple screening includes prospective and retrospective variants. Internal quality control of cytology screening largely depends on re-screening slides initially screened as negative or inadequate. Procedures may be designed to detect potential false negatives before final results are reported, in which case they have the potential to improve patient care as well as individual and laboratory accuracy. Procedures may also be designed to monitor accuracy of screening, either by measuring sensitivity and specificity of screening against the final result or by monitoring detection rates of cytological abnormalities.

The following re-screening procedures are proposed as contributing to the sensitivity of cytological screening or to general quality control:

- rapid reviewing of smears initially reported as negative or inadequate,
- rapid preview/pre-screening of all smears,
- random re-screening (full re-screening of a 10% random sample of smears reported as negative or unsatisfactory),
- targeted re-screening of specific patient groups,
- seeding abnormal cases into the screening pools,
- seeding abnormal cases into the re-screening pools,
- retrospective re-screening of negative cervical cytology specimens from patients with a current high-grade abnormality (targeted reviewing), and
- automated re-screening of smears initially reported as negative.

Rapid review (RR) consists of re-screening quickly, for 30 to 120 seconds, all slides that are originally reported as within normal limits or as inadequate in order to identify those that might

contain missed abnormalities. Those suspect smears are subsequently fully checked by an experienced cytotechnologist or cytopathologist who determines the final report.

- Rapid or partial reviewing of all smears has been introduced in the United Kingdom as an alternative and appears to be a useful quality control standard (Faraker & Boxer, 1996).
- In a recent study of published data on rapid reviewing of cervical smears, evidence was established that RR of all negative preparations results in the detection of more additional abnormalities in comparison to fully re-screening only 10 % of the negative workload (Arbyn & Schenck, 2000; Amaral *et al.*, 2005).

Rapid preview/pre-screening (RP) of all smears

RP is defined as partial microscopic inspection of a slide during a limited duration (maximum 120 seconds) before full routine examination.

- The essential difference between rapid pre-screening and rapid reviewing is that in RP all slides are submitted to a quick partial scanning by a cytotechnologist, while in rapid review only slides initially indicated as negative are reviewed (Arbyn *et al.*, 2003).
- The organisational advantage of RP is that it rapidly identifies most of the abnormal cases.
- The accuracy of rapid screening in picking up cytological lesions, relative to full routine screening can be easily computed.
- The process is not influenced by previous markings on the slide.
- Rapid pre-screening shows considerable promise as a quality control process, with a sensitivity gain comparable to that of rapid reviewing, and superior to that of 10% full re-screening (Arbyn *et al.*, 2003).

Random re-screening of a random fraction of smears reported as negative

- Random re-screening is widely practiced in the United States and suggested by some European countries (Cochand-Prollet *et al.*, 2004). CLIA '88 regulations specify that at least 10% of samples interpreted as negative have to be re-screened by a cytopathologist or a qualified supervisory cytotechnologist.
- Its value in detecting false-negative diagnoses has been criticised for its lack of efficiency and statistical power (Hutchinson, 1996; Melamed, 1996).

Targeted re-screening of specific patient groups selects smears from patients known to be at higher risk of having cytological abnormalities, and is done by a senior cytotechnologist or cytopathologist. The smears selected for targeted re-screening may be those with:

- a history of abnormal bleeding/spotting, e.g. intermenstrual, post coital, post menopausal,
- a history of recurrent cervical/vaginal infections,
- previous abnormal smears, or
- an abnormal cervix appearance on colposcopy.

Targeted re-screening is not standardised and its ability to detect additional lesions has not been compared to other methods such as random or rapid re-screening or pre-screening. Nevertheless, thought to be a good quality management method, it is practised in several European laboratories.

Automated re-screening

The potential benefit includes reduction of false-negative rates (Patten *et al.*, 1997); yet automated re-screening is an expensive approach for quality assurance (Kaminsky *et al.*, 1997).

Internal quality control based on screening detection and reporting rates

Monitoring primary screening detection rates

Accuracy of primary screening may be monitored without formal slide review procedures by measuring the percentages of the main types of cytological findings (high-grade, low-grade, inadequate, undetermined, negative) detected by individual screeners in comparison with the laboratory as a whole and local or national standards (Houliston *et al.*, 1998).

Monitoring pathologists' reporting rates

Pathologists' reporting rates for low-grade, high-grade and inadequate results form a useful guide to performance, which is important when the final pathologists' results are used as the outcome measure for primary screening performance.

Internal quality control based on correlation with clinical/histological outcome

Correlation of cytology with clinical outcome forms an important aspect of quality assurance and requires systems to be in place for ascertaining results of biopsies, colposcopy findings and other events.

Cyto-clinical correlation. Contact with clinicians and access to cancer registry data is essential.

- Laboratories should establish a mechanism to ensure follow-up of patients with cytology suggesting high-grade intraepithelial lesions and invasive carcinoma.
- Cyto-histological correlation is a major tool in internal education for both cytology and histology. The laboratory must have a clearly defined policy regarding the methods used for cyto-histologic correlation.
- The laboratory should compare all abnormal cytology reports with subsequent histopathology, if available, and determine the causes of any discrepancy.
- The correlation process should be documented in the laboratory quality assurance program.
- Positive predictive value for high-grade cytology provides a measure of accuracy of cytology reporting.

Cyto-virological correlation: If HPV testing can be used as a triaging test for patients with diagnosis of atypical squamous cells of undetermined significance (ASC-US), HPV positivity should be found in 30%, at least. See also Chapter 3.

Audit of interval cancers. Re-screening of smears from patients with negative or low-grade test results less than 3-5 years before the diagnosis of invasive cancer forms an important part of quality control, but should be taken in the context of all components of the screening history, including cytological screening errors, sampling errors, non-compliance with follow-up recommendations, incomplete treatment and whether or not the cancer was screen-detected. A link between the cancer registry and the cytology laboratory is a pre-requisite. Review of previous slides in women with invasive cancer should be carried out as far as possible in the context of the routine screening process. This means that slides should be re-screened alongside negative and/or positive controls and the labels concealed. More than one cytopathologist/cytotechnologist should review the slides, and preferably three. Review diagnoses should distinguish obvious false-negative interpretations from cytological features recognised as being at risk for being potential false negatives, such as few, small or pale abnormal cells (Mitchell & Medley, 1995; O'Sullivan *et al.*, 1998).

4.7.1.3 Internal continuing education

Encouraging communication and discussion of difficult cases between cytotechnologists and/or cytopathologists has a high impact on individual knowledge. Additionally:

- There should be a good supply of up-to-date cytology textbooks.
- The laboratory should have a subscription or online access to one or more of the cytology journals.
- Cytotechnologists and cytopathologists should participate in regular meetings on review cases.
- Performance evaluations should be used to identify those with deficiencies in knowledge and skills who would benefit from a more directed educational program.

4.7.2 External quality management

4.7.2.1 External continuing education

Although not mandatory under most regulations, external ongoing education should be an important component of any quality assurance program. Ongoing education is a requirement for proficiency in cytology. This requirement can be fulfilled by:

- attending workshops and symposia,
- regional inter-laboratory slide review sessions,
- participation in proficiency testing,
- teaching cytotechnology students, pathology residents and fellows, and
- independent study contributions to laboratory handbooks or work in committees of the relevant medical societies.

Inter-laboratory slide review sessions have been shown to increase reproducibility of cytology interpretation between participating laboratories (Ronco *et al.*, 2003).

Additionally, the ability of all persons involved in the screening process to work actively on their continuing education should be encouraged by the laboratory manager. Membership of regional, national or international societies for cytology should be seen as part of external continuing education. Cooperation with dedicated cytotechnologists from other labs improves motivation. Excellent motivation of many cytotechnologists is documented by their willingness to take voluntary proficiency tests. Therefore, staff should be given time away from their routine duties to allow them to take advantage of these procedures.

4.7.2.2 External quality control of screening skills

Proficiency testing is mandated in some but not all member states of the European Union. Proficiency testing, accreditation and recertification do not always go hand in hand.

- The International Academy of Cytology offers both proficiency testing and recertification based on continuing education credits earned via continued practise in cytology and participation in continuing education events (www.cytology-iac.org).
- The European Federation of Cytology Societies EFCS offers the EFCS aptitude test (QUATE test), which is based on the proficiency testing system used in the UK and widely accepted by Denmark and Italy (www.cytology-efcs.org).
- Voluntary proficiency tests should be designed to be educational, but procedures should be agreed beforehand for managing persistent poor performance.

- External quality assurance via test cases may take the form of regular examination of "test" cases, either as glass slides or electronic images, with assessment of individual performance on a voluntary basis (Cochand-Priollet *et al.*, 2004).
- Test slides should be designed to mimic normal practice, and the diagnoses should be agreed upon in advance by a central panel or, where relevant, confirmed by histology.

External quality assurance may also take the form of monitoring laboratory and personal reporting rates for high-grade and low-grade cytological abnormalities and comparing results with national standards (Breitenecker *et al.*, 2004). In the UK, reporting rates of all cytology laboratories are published annually and are used to provide achievable ranges for reporting cytological abnormalities (NHSCSP, 2000).

4.7.2.3 Accreditation of the laboratory unit

Based on predefined standards, an external organisation checks (Cooper & Hewison, 2002) and finally certifies the quality of the institution under investigation. Standards are documented agreements containing technical specifications or other precise criteria to be used consistently as rules or guidelines and definitions of characteristics, to ensure that materials, products, processes and services are fit for their purpose.

- External standards have to be distinguished from internal standards. While internal standards are a must for any quality management, the value of external standards is still under discussion (Klazinga, 2000; Moeller *et al.*, 2000; Burnett *et al.*, 2002). In Australia, standards for gynaecologic cytology are set up by the National Pathology Accreditation Advisory Board (Mody *et al.*, 2000).
- A variety of international/national accreditation agencies offer certification via external audits for laboratories. These private organisations have to be accredited by ministries of the different countries. *The International Organisation for Standardization (ISO)* is a worldwide non-governmental federation of national standards bodies from more than 140 countries, one from each country (www.iso.ch/). ISO's work results in international agreements that are published as International Standards (Klazinga, 2000).

Accreditation of the cytology laboratory is still voluntary in the majority of member states of the European Union. The Clinical Pathology Accreditation (UK) Ltd complies with the international standards ISO 17011 & ISO 9001: 2000. Submissions for cytology departments separate from combined histopathology/cytology departments will be not allowed in the future (www.cpa-uk.co.uk). Other countries have developed or are developing national or local accreditation programs for cytology laboratories (Klabunde *et al.*, 2002).

- In the case of accreditation, a minimum size of the cytology unit appears worthwhile. At least four persons should be involved in the screening process. There should be a minimum throughput of 15, 000 gynaecological slides per year.
- Re-certification should take place three years after the first accreditation, then every five years.

4.7.3 Responsibilities for quality control

The laboratory manager is responsible for the quality system and for the approval of working guidelines and procedures. See also Section 4.3.

4.8 Communication

4.8.1 Other laboratories

Laboratories should make relevant clinical information and follow-up data available to other laboratories taking part in the cervical screening program.

4.8.2 General practitioners, gynaecologists and other sample takers

Sample takers should be informed annually about their percentage of less-than-satisfactory or unsatisfactory cell samples versus the mean percentage of the country/region/laboratory.

Sample takers must provide the essential information using the standard request form.

Gynaecologists should make relevant clinical information and follow-up data available to laboratories taking part in the cervical screening program.

In certain areas, if a gynaecologist takes the smear, copies of the cervical smear results are sent to the woman's GP according to local inter-professional agreements.

4.8.3 Health authorities

Cytological and histological records must be sent at regular intervals to the regional or national screening or cancer registry that is responsible for the monitoring of screening programmes. This condition should be mandatory and should include all records irrespective of indication for the examination, status of the woman, the smear taker or the laboratory. Laboratories should receive reports with the results of process and impact evaluation of screening.

The screening registry can also provide specific and general statistics to participating laboratories.

4.8.4 Patients

Depending on regional or national legal practice, informing the woman of the result of the smear is the responsibility of the sample taker or of the laboratory.

4.9 References

- Amaral R.G., Zeferino L.C., Hardy E., Westin M.C., Martinez E.Z., & Montemor E.B. (2005). Quality assurance in cervical smears: 100% rapid re-screening vs. 10% random re-screening. *Acta Cytol.* **49**: 244-248.
- Arbyn M. & Schenck U. (2000). Detection of false negative Pap smears by rapid reviewing: a meta-analysis. *Acta Cytol.* **44**: 949-957.
- Arbyn M., Schenck U., Ellison E., & Hanselaar A. (2003). Metaanalysis of the accuracy of rapid pre-screening relative to full screening of pap smears. *Cancer* **99**: 9-16.
- Arcelay A., Sanchez E., Hernandez L., Inclan G., Bacigalupe M., Letona J., Gonzalez R.M., & Martinez-Conde A.E. (1999). Self-assessment of all the health centres of a public health service through the European Model of total quality management. *Int J Health Care Qual Assur Inc Leadersh Health Serv* **12**: 54-58.
- Baker A. & Melcher D.H. (1991). Rapid cervical cytology screening. *Cytopathology* **2**: 299-301.
- Baker R.W., Wadsworth J., Brugal G., & Coleman D.V. (1997). An evaluation of 'rapid review' as a method of quality control of cervical smears using the AxioHOME microscope. *Cytopathology* **8**: 85-95.
- Bekova A. & Kobilkova J. (2005). Cytopathology in the Czech Republic. *Cytopathology* **16**: 147-149.
- Breitenecker G., Dinges H.P., Regitnig P., Wiener H., & Vutuc C. (2004). Cytopathology in Austria. *Cytopathology* **15**: 113-118.
- Burnett D., Blair C., Haeney M.R., Jeffcoate S.L., Scott K.W., & Williams D.L. (2002). Clinical pathology accreditation: standards for the medical laboratory. *J Clin Pathol* **55**: 729-733.
- Cochand-Priollet B., Vincent S., & Vielh P. (2004). Cytopathology in France. *Cytopathology* **15**: 163-166.
- Cooper J. & Hewison A. (2002). Implementing audit in palliative care: an action research approach. *J Adv Nurs* **39**: 360-369.
- Council of Europe (2001). Developing a methodology for drawing up guidelines on best medical practice (recommendation (2001) 13 and explanatory memorandum).
- Drijkoningen M., Bogers J.P., Bourgain C., Cuvelier C., Delvenne P., Gompel C., Saerens L., Thienpont L., Van Damme B., Van Eycken L., Verhest A., & Weynand B. (2005). Cytopathology in Belgium. *Cytopathology* **16**: 100-104.
- Faraker C.A. & Boxer M.E. (1996). Rapid review (partial re-screening) of cervical cytology. Four years experience and quality assurance implications. *J Clin Pathol* **49**: 587-591.
- Gill G.W., Frost J.K., & Miller K.A. (1974). A new formula for a half-oxidized hematoxylin solution that neither overstains nor requires differentiation. *Acta Cytol.* **18**: 300-311.

Grenko R.T., Abendroth C.S., Fraumeni E.E., Ruggiero F.M., & Zaino R.J. (2000). Variance in the interpretation of cervical biopsy specimens obtained for atypical squamous cells of undetermined significance. *Am.J.Clin.Pathol.* **114**: 735-740.

Houliston D.C., Boyd C.M., Nicholas D.S., & Smith J.A. (1998). Personal performance profiles: a useful adjunct to quality assurance in cervical cytology. *Cytopathology* **9**: 162-170.

Hutchinson M.L. (1996). Assessing the costs and benefits of alternative re-screening strategies. *Acta Cytol.* **40**: 4-8.

Kaminsky F.C., Benneyan J.C., & Mullins D.L. (1997). Automated re-screening in cervical cytology. Mathematical models for evaluating overall process sensitivity, specificity and cost. *Acta Cytol.* **41**: 209-223.

Klabunde C.N., Sancho-Garnier H., Taplin S., Thoresen S., Ohuchi N., & Ballard-Barbash R. (2002). Quality assurance in follow-up and initial treatment for screening mammography programs in 22 countries. *Int J Qual Health Care* **14**: 449-461.

Klazinga N. (2000). Re-engineering trust: the adoption and adaption of four models for external quality assurance of health care services in western European health care systems. *Int J Qual Health Care* **12**: 183-189.

Klinkhamer P.J., Vooijs G.P., & De Haan A.F. (1988). Intraobserver and interobserver variability in the diagnosis of epithelial abnormalities in cervical smears. *Acta Cytol.* **32**: 794-800.

Koss L.G. (1989). The Papanicolaou test for cervical cancer detection. A triumph and a tragedy. *JAMA* **261**: 737-743.

Koss L.G. (1992) Diagnostic Cytology and its histopathologic basis., 4th edn.

Melamed M.R. (1996). Re-screening for quality control in cytology. *Acta Cytol.* **40**: 12-13.

Mitchell H. & Medley G. (1995). Differences between Papanicolaou smears with correct and incorrect diagnoses. *Cytopathology* **6**: 368-375.

Mody D.R., Davey D.D., Branca M., Raab S.S., Schenck U., Stanley M.W., Wright R.G., Arbyn M., Beccati D., Bishop J.W., Collaço L.M., Cramer S.F., Fitzgerald P., Heinrich J., Jhala N.C., Montanari G., Kapila K., Naryshkin S., & Suprun H.Z. (2000). Quality assurance and risk reduction guidelines. *Acta Cytol.* **44**: 496-507.

Moeller J., Breinlinger-O'Reilly J., & Elser J. (2000). Quality management in German health care--the EFQM Excellence Model. *Int J Health Care Qual Assur Inc Leadersh Health Serv* **13**: 254-258.

NHSCSP. Achievable standards, benchmarks for reporting, and criteria for evaluating cervical cytopathology. Second edition including revised performance indicators (2nd edition). NHSCSP publication 1, 1-36. 2000. Sheffield, NHS Cancer Screening Programmes

O'Sullivan J.P., A'Hern R.P., Chapman P.A., Jenkins L., Smith R., Al-Nafussi A., Brett M.T., Herbert A., McKean M.E., & Waddell C.A. (1998). A case-control study of true positive versus false negative cervical smears in women with cervical intraepithelial neoplasia (CIN) III. *Cytopathology* **9**: 155-161.

O'Sullivan J.P., Ismail S.M., Barnes W.S., Deery A.R., Gradwell E., Harvey J.A., Husain O.A., Kocjan G., McKee G., Olafsdottir R., Ratcliffe N.A., & Newcombe R.G. (1996). Inter- and intra-observer

variation in the reporting of cervical smears: specialist cytopathologists versus histopathologists. *Cytopathology* **7**: 78-89.

Papanicolaou G.N. (1942). A new procedure for staining vaginal smears. *Science* **95**: 438-439.

Patten S.F., Lee J.S.J., Wilbur D.C., Bonfiglio T.A., Colgan T.J., Richart R.M., & Moinuddin S. (1997). The Autopap 300 QC system multicenter clinical trials for use in quality control re-screening of cervical smears. I. A prospective intended use study. *Cancer* **81**: 337-342.

Ronco G., Montanari G., Confortini M., Parisio F., Berardengo E., Delpiano A.M., Arnaud S., Campione D., Baldini D., Poll P., Lynge E., Mancini E., & Segnan N. (2003). Effect of circulation and discussion of cervical smears on agreement between laboratories. *Cytopathology* **14**: 115-120.

Schenck U., Engelhardt W., Hinrichs F., Jordan B., Müller-Wallraf R., Tenberken K., & Witting C. (1998). Leitlinie zur Zertifizierung von zytologisch tätigen Assistenten/innen in der Gynäkologischen Zytologie durch die Deutsche Gesellschaft Für Zytologie (DGZ).

Schenck U. & Planding W. (1996). Quality assurance by continuous recording of the microscope status. *Acta Cytol.* **40**: 73-80.

Smith P.A. & Hewer E.M. (2003). Examination for the Certificate in Advanced Practice in Cervical Cytology--the first year's experience. *Cytopathology* **14**: 101-104.

Stoler M.H. (2002). Toward objective quality assurance: the eyes don't have it. *Am.J.Clin.Pathol.* **117**: 520-522.

Stoler M.H. & Schiffman M.A. (2001). Interobserver reproducibility of cervical cytologic and histologic interpretations. *JAMA* **285**: 1500-1505.

Tarkkanen J., Geagea A., Nieminen P., & Anttila A. (2003). Quality improvement project in cervical cancer screening: practical measures for monitoring laboratory performance. *Acta Obstet Gynecol Scand* **82**: 82-88.

Valkov I., Zlatkov V., & Kostova P. (2004). Cytopathology in Bulgaria. *Cytopathology* **15**: 228-232.

5

Techniques and quality assurance guidelines for histopathology

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5.1 Executive summary

Histopathology provides the final diagnosis on the basis of which treatment is planned, and serves as the gold standard for quality control of cytology and colposcopy. It is also the source of the diagnostic data stored at the cancer registry and used for evaluation of screening programmes. It is therefore important that histopathology standards are monitored and based on agreed diagnostic criteria.

Histology is required to diagnose the degree of abnormality in women with persistent low-grade abnormalities including HPV-lesions, as well as high-grade lesions. Cytology may also suggest either glandular abnormalities or be suggestive of high-grade CIN, AIS or invasive cancer. Histopathologists should be aware of, and familiar with, the nature of cytological changes which may be relevant to their reports. The accuracy of the histopathological diagnosis of tissue specimens depends on adequate samples, obtained by colposcopically directed punch biopsies (with endocervical curettage if necessary) or excision of the transformation zone or conisation. An accurate histological diagnosis further depends on appropriate macroscopic description, technical processing, microscopic interpretation and quality management correlating cytological and histological diagnosis.

This chapter proposes guidelines for sampling and processing of cervical tissue specimens obtained by biopsy, excision and/or curettage.

5.2 Introduction

Cervical cytology currently represents the primary screening method, but does not provide the final diagnosis. Abnormal cervical cytology should be followed by colposcopy and microscopic evaluation of cervical tissue (Costa *et al.*, 1991).

Adequate colposcopy is necessary to locate the most abnormal areas of the cervix (Singer & Monaghan, 2000). The criteria for colposcopic referral and the requirements for high-quality colposcopy are described in Chapter 6. The validity of the histopathological report will also depend on the quality of the biopsy. Since these specimens are often very small (in the range of millimeters), careful handling and work-up is required.

If positive cytology does not correlate with the histological findings from the biopsies, the pathologist has to consider that a dysplastic lesion could be small and missed by the biopsy or alternatively not visible due to endocervical localisation. For this reason histology and cytology should be closely correlated to give the gynaecologist a clear impression of the individual situation.

Excision biopsy represents a special type of tissue specimen. Its objective is the complete removal of dysplastic lesions found by a previous biopsy and/or cytology. The histopathological report of an excision biopsy should include a clear diagnosis of the primary lesion and a description of the resection margins. Since possible microinvasion has a major impact on the management of patients, complete work-up of excised tissue in step serial sections is recommended. Additional immunohistochemistry in selected cases might support a diagnosis of possible microinvasion or vessel involvement and might help in the distinction between squamous or glandular neoplasia (Obermair *et al.*, 1998; Birner *et al.*, 2001).

5.3 Punch biopsies

Punch biopsies are small pieces of tissue a few millimetres in diameter that are removed from the cervical mucosa with a biopsy forceps. For indications, see Chapter 6.

5.3.1 Diagnostic goal

When colposcopy is satisfactory and obvious area(s) of CIN can be visualised, histological examination of punch biopsies can be sufficient to obtain a correct diagnosis.

5.3.2 Macroscopic description

The number, diameter, color and consistency of the specimens should be documented.

5.3.3 Technique

In case of multiple cervical biopsies, each area of the cervix from which the biopsies have been taken should be identified separately. Specimens are fixed in 4% buffered formalin at room temperature, followed by paraffin embedding according to routine procedures. Four μm paraffin serial tissue sections are stained for H&E and/or processed for special stains and immunohistochemistry, if indicated.

5.3.4 Histological diagnosis

The histological report should include:

- Tissue type
- Absence or presence and type of neoplastic lesions
- Grade of identified lesions:
- Squamous lesions: cervical intraepithelial neoplasia 1-3 (CIN1-3), invasive cancer
- Glandular lesions: high-grade and low-grade cervical glandular intraepithelial neoplasia (CGIN) invasive adenocarcinoma or adenosquamous carcinoma
- Presence of HPV-associated changes (koilocytes, dyskeratosis)
- Size of the lesion (in mm)
- Characterization of non-neoplastic lesions
- Stromal reaction: presence and extent of inflammation or desmoplastic reaction

- In case of invasive cancer, depth of invasion, presence of lymphovascular involvement and the degree of differentiation should be documented.¹

These guidelines strongly recommend the CIN classification for histological diagnosis. Careful attention to criteria for diagnosis of the three grades of CIN (CIN1-3) should be observed (WHO, 2003). CIS is usually combined with CIN3 (in the UK both are recorded as "in-situ carcinoma of the uterine cervix" in the national cancer registry).

Broadly speaking CIN1 / koilocytosis (correlating to LSIL) is likely to be reversible and associated with productive HPV infection (Mitchell MF, 1996). CIN2 and CIN3 / carcinoma in situ (correlating to HSIL) are more likely to persist or progress if left untreated and also more likely to be associated with HPV integrated into the host genome (Mitchell MF, 1996). Two meta-analyses of follow-up studies indicate a greater likelihood of regression and a lesser likelihood of progression of CIN2 compared to CIN3 (Ostor, 1993; Melnikow, 1998). CIN3 is a more robust diagnosis than CIN2 and is therefore more useful as a gold standard for outcome.

In small biopsies it may occasionally be necessary to report CIN as "ungraded" but where possible diagnoses such as CIN1-2 should be avoided.

The distinction between individual grades of CIN is poorly reproducible but improves with increasing grade. Diagnoses of CIN3 and invasive cancer are the most reproducible (Ismail *et al.*, 1989; Stoler & Schiffman, 2001). Immature squamous metaplasia and atrophic squamous epithelium are documented sources of misinterpretation and may be mistaken for CIN1-2 (Crum *et al.*, 1983). In such cases p16 staining and repeat biopsy after oestrogen may be helpful (Klaes *et al.*, 2002, see also section 5.6).

Precise grading of CGIN is poorly reproducible and there is little evidence that it forms a biological spectrum (WHO, 2003). High-grade CGIN equates to adenocarcinoma in situ and low-grade CGIN is usually managed in the same way. Low-grade CGIN should be reported infrequently and care must be taken to distinguish it from benign conditions that may mimic it (NHSCSP Publication No 10, April 1999). The same strictures apply to diagnoses of glandular dysplasia and atypia (WHO, 2003, Goldstein, 1998).

¹ **CIN1 (flat condyloma; koilocytosis; mild dysplasia):** Neoplastic, basaloid cells and mitotic figures occupy the lower third of the epithelium in CIN1 lesions. These lesions frequently show marked HPV cytopathic effects including perinuclear halos, multinucleation and nuclear membrane irregularities, and hyperchromasia (e.g., "koilocytosis").

CIN2 (moderate dysplasia): In CIN2, neoplastic basaloid cells and mitotic figures occupy the lower two thirds of the epithelium.

CIN3 (severe dysplasia lumped with carcinoma in situ): The characteristic histological feature of CIN3 is the presence of neoplastic basaloid cells and mitotic figures that occupy the full thickness of the epithelium. These cells have high nuclear:cytoplasmic ratios, with scant cytoplasm and dense, hyperchromatic nuclei having coarse clumped chromatin and irregular nuclear outlines (IARC, 2005).

² **CGIN** is recognised histologically by a combination of architectural and cytological abnormalities, though a consistent feature is the presence of nuclear abnormalities. Not all features are seen in every case. Architectural features include glandular crowding, branching and budding; intraluminal papillary projections; cribriform pattern. Cytological features include abrupt junction between normal and abnormal epithelium; intestinal / goblet cell metaplasia; loss of mucin-secretion in cells of endocervical type; cellular stratification but only when combined with nuclear changes; loss of nuclear polarity; nuclear enlargement, pleomorphism, hyperchromasia; mitotic activity, some of which may be abnormal forms; prominent nucleoli; apoptosis. It can usually be distinguished from microinvasive adenocarcinoma by its limitation to the glandular field, admixture of normal and abnormal glands, lack of stromal response and lack of cytological changes seen in microinvasive adenocarcinoma (increased pleomorphism, paler, more copious and eosinophilic cytoplasm). Invasion should not be excluded on small punch biopsies

5.4 Excision biopsies

Excision biopsies represent nearly cone-shaped portions of cervical tissue including the lower part of the endocervical canal and a portion of the ecto-cervix. Excision biopsies include cold knife conisation, laser conisation and Large Loop Excision of the Transformation Zone (LLETZ²). Cold knife (and laser) cone biopsies are indeed cone-shaped tissue specimens whereas LLETZ excisions in most cases represent a more disc shaped, ectocervical portion sometimes with an extra biopsy from the middle of the endocervical canal (top hats, Mexican hats). The histopathologist should be able to recognize and deal with these different forms of excision biopsies. For technical details of excision and clinical indications, see Chapter 6.

5.4.1 Diagnostic goals

An excision biopsy should aim to remove all pathological tissue (identified by colposcopy) including a part of the endocervical canal and the transformation zone. The procedure should be diagnostic (provide a precise histological diagnosis) and therapeutic (resection of the lesion in toto).

5.4.2 Macroscopic description

Description should include the size of the specimen (length and diameter), localisation of the cervical canal (central, paracentral or marginal), any visible lesion, and the position of any markings and sutures for orientation of the specimen (Horn *et al.*, 1999).

5.4.3 Technique

Usually an excision biopsy removes the whole transformation zone, including a portion of the lower endocervical canal.

The biopsy should be clearly marked (e.g. colour or threads at 12 o'clock) to enable adequate orientation throughout the future workup (Robboy *et al.*, 1994; Robboy *et al.*, 2002). The integrity of the cervical canal should be preserved and not altered by prior dilatation.

There exist various techniques for sectioning excision biopsies (Heatley, 2001). The methods used include opening, pinning and serially sectioning the specimen – or fixing and serially sectioning the unopened specimen at right angles to the os. A simple and easily reproducible method is the

² In American terminology most often the term LEEP (Lus Electrosurgical Procedure) is used, whereas in the English literature, usually the term LLETZ (Large Loop Excision of the Transformation Zone) is used. In this guideline only LLETZ is used.

division of the tissue into two equal halves along the axis of the cervical canal. Each half is embedded in-separate deep (1 cm) cups followed by complete step (0.1 mm) serial sectioning. This method is described in the guidelines of the Austrian Society of Pathology (Österreichische Gesellschaft für Pathologie, 2000) and results in histological slides that are easy to orient and interpret, including inmost cases an accurate evaluation of the resection margins (see Fig.s 1 and 2).

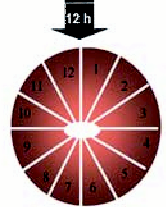
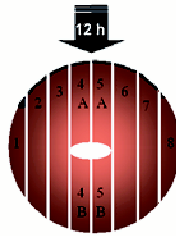
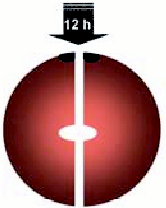
	Radial cutting includes opening longitudinally and pinning. Sequential identification of each section allows accurate mapping of the lesion. 
	Parallel antero-posterior cutting from left to right (or vice versa) should include ink application of one margin in minimum, application of multiple colour inks simplifies proper identification of various margins. If divided into an anterior and posterior fraction numbering of the posterior part should follow the same order as the anterior part.
	Division into two equal halve along the axis of the cervical canal. Each half should be marked by colour inking of one margin as a minimum, and is then embedded in separate deep (1 cm) cups followed by complete step (0.1 mm) serial sectioning.

Fig. 1. Examples of techniques for sectioning excision biopsies (graphics created by H. G. Wiener)

5.4.4 Histological diagnosis

Histological reports on an excision biopsy should provide a well defined pathological diagnosis as summarized below. The diagnosis should also be in concordance with the WHO histological classification of tumours of the uterine cervix (Tables 1 and 2). In addition to a precise description of the histological type of the lesion the report should include information concerning the

- Grade of neoplastic lesion
- Localization of the lesion within the excision biopsy
- Uni/multifocality of the lesion
- Extent of the lesion (in cases of microinvasive and invasive cancer, measurement of vertical and horizontal diameters is crucial for adequate staging).
- Stromal reaction
- Involvement of microvessels
- Relation of tumor tissue to all resection margins (distance)
- Description and characterization of additional non-neoplastic lesions (tuboendometroid metaplasia, microglandular hyperplasia, endometriosis, regenerative and repair changes)

Table 1. Histological classifications of preinvasive intraepithelial lesions of the uterine cervix

Dysplasia classification	Cervical intraepithelial neoplasia (CIN and CGIN) classification	Bethesda classification (used for cytology)
Mild dysplasia	CIN 1	LGSIL
Moderate dysplasia	CIN 2	HGSIL
Severe dysplasia	CIN 3	HGSIL
Carcinoma in situ	CIN 3	HGSIL
Endocervical dysplasia	CGIN (low-grade, high-grade)	AGC
Adenocarcinoma in situ	CGIN (high-grade)	AIS

The term microinvasive carcinoma may be applied to squamous cell carcinomas and adenocarcinomas but only when accompanied by measurements of depth and lateral extent of a completely excised lesion. The diagnosis can then be defined according to the FIGO definitions of stage 1A1 and 1A2 (Table 3), for which there is an evidence base for outcome after treatment (WHO 2003). Depth of invasion should be measured from the base of the epithelium from which the invasive lesion arises and the lateral extent from the section in which the width is widest. Stage 1A1 lesions (less than 3 mm depth and less than 7 mm width) should be specified as either one or more foci of early stromal invasion or a confluent lesion. Stage 1A2 lesions are defined as 3 – 5 mm depth and less than 7 mm width.

Adenocarcinomas should be measured and recorded in the same way but there are no reliable criteria for distinguishing 1A1 and 1A2 tumours.

If an invasive lesion cannot be measured as indicated above, it should be described as a small invasive carcinoma and classified as 1B1. The presence of lymphovascular invasion should be recorded but does not affect the FIGO stage.

5.5 Endocervical curettage (ECC)

Endocervical curettage (ECC) is a sampling procedure to obtain endocervical tissue. Readers are referred to Chapter 6 for the clinical indications of ECC.

5.5.1 Diagnostic goal

The objective of ECC is:

- to evaluate any ectocervical squamous cell lesion extending to the endocervical canal;
- to detect endocervical adenocarcinoma and its precursor lesions; and
- to determine cervical involvement of any non-cervical malignancies.

Endocervical curettage combined with colposcopically directed ectocervical punch biopsies allows histological assessment of both the ecto- and endo-cervix, without excising a substantial amount of cervical tissue (Kobak *et al.*, 1995). Nevertheless, it should be kept in mind that ECC has a limited sensitivity to detect endocervical CIN or CGIN. Furthermore, ECC alters the architecture of the endocervical canal, compromising the assessment of a later conisation. Collection of endocervical cells, using an endocervical brush, has in several studies shown a higher sensitivity (but a lower specificity) than ECC (Hoffman *et al.*, 1993; Mogensen *et al.*, 1997; Boardman *et al.*, 2003). Other authors support the use of ECC, since it allows the detection of colposcopically hidden lesions (Pretorius *et al.*, 2004).

5.5.2 Macroscopic description

The number, diameter, color and consistency of the specimen fragments should be documented.

5.5.3 Technique

ECC provides tissue from the endocervical canal by using an endocervical curette. Tissue from all four sides of the cervical canal should be obtained.

Very small specimens should be wrapped in paper prior to paraffin embedding.

Serial sections of the biopsy specimens are recommended.

5.5.4 Histological diagnosis

The description of tissues found in the curetted material should specify:

- The presense of endocervical glands, endometrial tissue, squamous epithelium;
- Glandular or squamous intraepithelial neoplastic and non-neoplastic changes;
- Evidence for invasion;
- Neoplastic or non-neoplastic stromal alterations; and
- Presence and kind of inflammatory processes.

5.6 Immunohistochemistry

Immunohistochemistry might be helpful, if H&E stained sections do not provide enough information for inclusion or exclusion of intraepithelial or invasive neoplasia. Immunohistochemical staining of dysplastic lesions of the cervix with a variety of antibodies to cell cycle-associated proteins can provide additional information in those difficult cases.

Proliferation markers are widely used by pathologists and can be easily applied on formaline-fixed and routinely-processed cervical tissues.

- The Ki-67 antigen is a non-histone protein expressed in the nucleus in all phases of the cell cycle except G0. The most commonly used monoclonal antibody for immunohistochemical detection of the Ki-67 antigen in paraffin sections is clone MIB1. The extent of Ki-67 immunostaining generally parallels increasing grades of dysplasia (Bulten *et al.*, 1996). Moreover, expression of Ki-67 allows distinction of atrophic cervical epithelium (negative for Ki-67) from neoplastic or dysplastic cervical epithelium (positive for Ki-67) (Bulten *et al.*, 2000).
- The proliferating cell nuclear antigen (PCNA), identified as a polymerase-associated protein and is synthesized in early G1 and S phases of the cell cycle and might be also helpful (Smela *et al.*, 1996).

Cervical neoplasia, but not other cervical epithelia, expresses high levels of the cyclin-dependent kinase inhibitor p16, suggesting that staining for this marker could provide diagnostic support to distinguish true CIN/dysplasia from immature metaplasia or other non-neoplastic changes of the cervix. Immuno-detection of p16 in dysplastic epithelium using monoclonal antibodies in routinely processed histological cervical tissue was recently described by Klaes *et al.* (2001).

Other immunohistochemical markers like antibodies directed to extracellular matrix components of the basal membrane could be used for the assessment of possible microinvasion in selected cases. Several studies have shown that routine H&E slides are not always adequate for detection of vascular invasion, especially in cases with strong inflammatory stromal reaction. Antibodies against endothelial marker proteins, e.g. Factor VIII-related antigen, stain both lymphatic and blood vessel endothelium and therefore represent a useful tool for the detection of lymphovascular invasion in cervical cancer. For a more selective assessment of blood vessels, CD31 can be recommended. For detection of lymph vessel involvement, immunostaining with newly recognized lymphendothelial proteins (like podoplanin) can be performed (Obermair *et al.*, 1998; Birner *et al.*, 2001).

5.7 Data collection

Laboratories should provide a standard request form for collaborating gynaecologists that includes administrative patient data, previous reports of cytology, colposcopy, and cervical/uterine/vaginal/vulva histology. Indication for the intervention and the type of biopsy (punch, LLETZ, cone, ECC, endocervical brushing) must be stated clearly.

Computerized documentation of histological reports and adequate storage of paraffin blocks and sections (slides) must follow the local legal requirements for data protection. Often blocks and slides are kept indefinitely, the principle being to hold them for at least the lifetime of the patient.

At a minimum, data should include:

- patients' key data;
- date of request;
- specification of material; and
- a detailed summary histological report, that is coded following a recognised international standard for histological classification (such as SNOMED, CIN/CGIN).

Histological data should be communicated to the national or regional screening register in order to correlate data as explained in Chapter 2. Linkage of histological outcomes with screening histories,

within the laboratory or in collaboration with the screening register, should allow the creation of cyto-histological cross tables and assessment of the predictive value of cytology (see Chapter 7). Archived Pap smears and histological blocks of cervical tissue constitute a very important source for bio-bank research. The EU is currently promoting systems allowing high-quality research using stored human biological material (<http://www.cancerbiobank.org/>).

5.8 Quality assurance

All personnel involved in the histological part of the cervical screening process should understand each step of the entire work-up procedure. Internal process-oriented quality assessment should include a laboratory handbook, safety instructions and protocols (Vutuc *et al.*, 1999); see also Chapter 4. Histological reports should allow comparison and correlation with cytology and colposcopy.

Regular internal meetings for technical troubleshooting, training and diagnostic discussion should complete the working procedure. Additionally, interdisciplinary meetings of pathologists, cyto-technicians and gynaecologists with discussion of cytological slides, colposcopic images and histological slides are recommended.

The readers are referred to Chapter 4 for details concerning continuing education and external quality control in cytopathology.

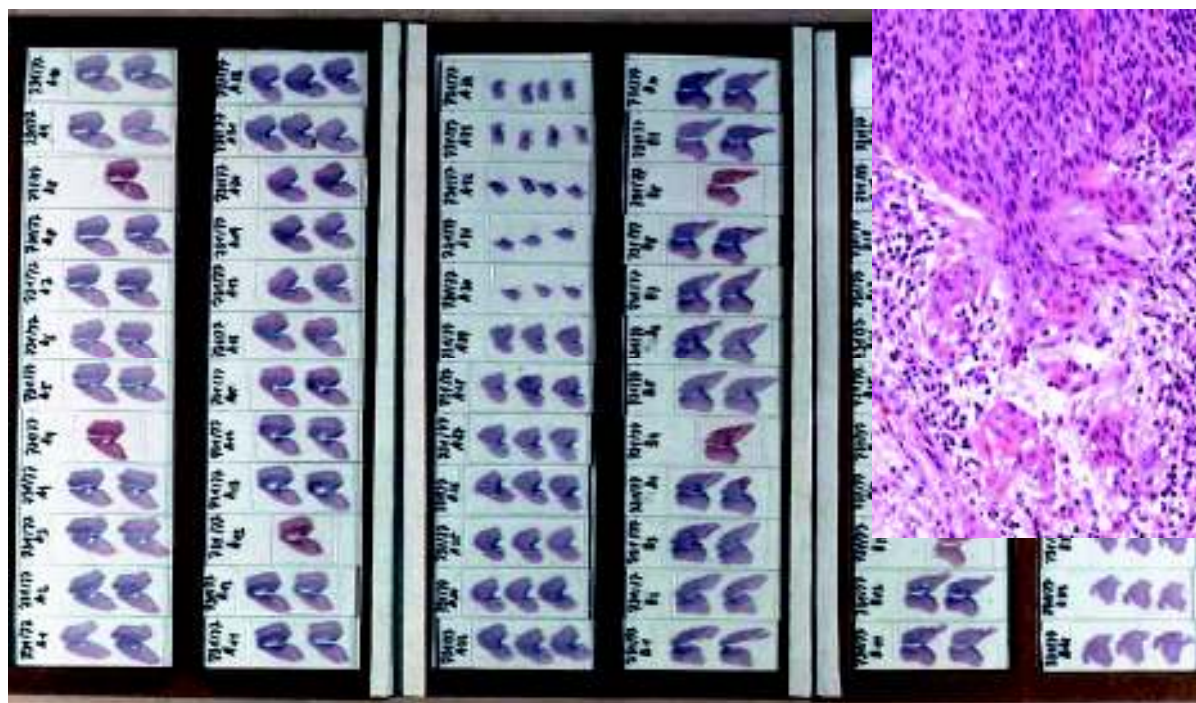


Fig 2. Serial sections of a cone biopsy for detection of microinvasive carcinoma (inset)

Table 2. WHO histological classification of malignant tumours of the uterine cervix¹**Epithelial tumours**

Squamous tumours and precursors

Squamous cell carcinoma, not otherwise specified

Keratinizing

Non-keratinizing

Basaloid

Verrucous

Warty

Papillary

Lymphoepithelioma-like

Squamotransitional

Early invasive (microinvasive) squamous cell carcinoma

Squamous intraepithelial neoplasia

Cervical intraepithelial neoplasia (CIN) 3/Squamous cell carcinoma in situ

Benign squamous cell lesions

Condyloma acuminatum

Squamous papilloma

Fibroepithelial polyp

Glandular tumours and precursors

Adenocarcinoma

Mucinous adenocarcinoma

Endocervical

Intestinal

Signet ring cell

Minimal deviation

Villoglandular

Endometroid adenocarcinoma

Clear cell adenocarcinoma

Serous adenocarcinoma

Mesonephric adenocarcinoma

Early invasive adenocarcinoma

Adenocarcinoma in situ

Glandular dysplasia

Benign glandular lesions

Müllerian papilloma

Endocervical polyp

Other epithelial tumours

Adenosquamous carcinoma

Glassy cell carcinoma variant

Adenoid cystic carcinoma

Adenoid basal carcinoma

Neuroendocrine tumours

Carcinoid

Atypical carcinoid

Small cell carcinoma

¹ Table reprinted from *Pathology and Genetics of Tumours of the Breast and Female Genital Organs (2003)*. World Health Organization Classification of Tumours, IARC Press, Lyon with permission from IARC.

Large cell neuroendocrine carcinoma
Undifferentiated carcinoma

Table 2. (continued)

Mesenchymal tumours and tumour-like conditions

Leiomyosarcoma
Endometroid stromal sarcoma, low-grade
Undifferentiated endocervical sarcoma
Sarcoma botroides
Alveolar soft part sarcoma
Angiosarcoma
Malignant peripheral nerve sheath tumour
Leiomyoma
Genital rhabdomyoma
Postoperative spindle cell nodule

Mixed epithelial and mesenchymal tumours

Carcinosarcoma (malignant Müllerian mixed tumour, metaplastic carcinoma)
Adenosarcoma
Wilms tumour
Adenofibroma
Adenomyoma

Melanocytic tumours

Malignant melanoma
Nevus cell nevus

Miscellaneous tumours

Tumours of germ cell type
Yolk sac tumour
Dermoid cyst
Mature cystic teratoma

Lymphoid and haematopoietic tumours

Malignant lymphoma
Leukemia

Secondary tumours

Table 3. TNM categories and FIGO staging

TNM	Explanation applicable to both systems	FIGO
Tx	Primary tumour cannot be assessed	
T0	No evidence of primary tumour	
Tis	Carcinoma in situ (preinvasive carcinoma)	0
T1	Cervical carcinoma confined to uterus (extension to uterus should be disregarded)	I
T1a	Invasive carcinoma diagnosed only by microscopy	IA
T1a1	Stromal invasion no greater than 3.0 mm in depth and 7.0 mm or less in horizontal spread	IA1
T1a2	Stromal invasion more than 3.0 mm and not more than 5.0 mm with horizontal spread 7.0 mm or less	IA2
T1b	Clinically visible lesion confined to the cervix or microscopic lesion greater than T1a2/1A2	IB
T1b1	Clinically visible lesion 4.0 cm or less in greatest dimension	IB1
T1b2	Clinically visible lesion more than 4.0 cm in greatest dimension	IB2
T2	Tumour invades beyond uterus but not to pelvic wall or to lower third of vagina	II
T2a	Without parametrial invasion	IIA
T2b	With parametrial invasion	IIB
T3	Tumour extends to pelvic wall, involves lower third of vagina, or causes hydronephrosis or non-functioning kidney	III
T3a	Tumour involves lower third of vagina, no extension to pelvic wall	
T3b	Tumour extends to pelvic wall or causes hydronephrosis or non-functioning kidney	
T4	Tumour invades mucosa of bladder or rectum or extends beyond true pelvis	IVA
M1	Distant metastasis	IVB

For details see L.H. Sobin, Ch. Wittekind (eds.): TNM Classification of Malignant Tumours. Sixth edition 2002, Wiley-Liss, Inc. and WHO 2003.

5.9 References

- Birner P., Obermair A., Schindl M., Kowalski H., Breitenecker G., & Oberhuber G. (2001). Selective immunohistochemical staining of blood and lymphatic vessels reveals independent prognostic influence of blood and lymphatic vessel invasion in early-stage cervical cancer. *Clin Cancer Res.* **7**: 93-97.
- Boardman L.A., Meinz H., Steinhoff M.M., Heber W.W., & Blume J. (2003). A randomized trial of the sleeved cytobrush and the endocervical curette. *Obstet. Gynecol.* **101**: 426-430.
- Bulten J., de Wilde P.C., Schijf C., van der Laak J.A., Wienk S., Poddighe P.J., & Hanselaar A.G. (2000). Decreased expression of Ki-67 in atrophic cervical epithelium of post-menopausal women. *J. Pathol.* **190**: 545-553.
- Bulten J., van der Laak J.A., Gemmink J.H., Pahlplatz M.M., de Wilde P.C., & Hanselaar A.G. (1996). MIB1, a promising marker for the classification of cervical intraepithelial neoplasia. *J. Pathol.* **178**: 268-273.
- Costa M.J., Grimes C., Tackett E., & Naib Z.M. (1991). Cervicovaginal cytology in an indigent population. Comparison of results for 1964, 1981 and 1989. *Acta Cytol.* **35**: 51-56.
- Crum C.P., Egawa K., Fu Y.S., Lancaster W.D., Barron B., Levine R.U., Fenoglio C.M., & Richart R.M. (1983). Atypical immature metaplasia (AIM). A subset of human papilloma virus infection of the cervix. *Cancer* **51**: 2214-2219.
- Goldstein N.S., Ahmad E., Hussain M., Hankin R.C., Perez-Reyes N. (1998). Endocervical glandular atypia: does a preneoplastic lesion of adenocarcinoma in situ exist? *Am. J. Clin. Pathol.* **110**:200-209.
- Heatley M.K. (2001). How many histological levels should be examined from tissue blocks originating in cone biopsy and large loop excision of the transformation zone specimens of cervix? *J Clin Pathol* **54**: 650-651.
- Hoffman M.S., Sterghos S.Jr., Gordy L.W., & Gunasekar D. (1993). Evaluation of the cervical canal with the endocervical brush. *Obstet. Gynecol.* **82**: 573-577.
- Horn L.C., Riethdorf L., & Loning T. (1999). Leitfaden für die Präparation uteriner Operationspräparate. *Pathologe* **20**: 9-14.
- IARC (2005). Cervix Cancer Screening. IARC Handbooks of Cancer Prevention. Vol. 10. IARCPress, Lyon.
- Ismail S.M., Colclough A.B., Dinnen J.S., Eakins D., Evans D.M., Gradwell E., O'Sullivan J.P., Summerell J.M., & Newcombe R.G. (1989). Observer variation in histopathological diagnosis and grading of cervical intraepithelial neoplasia [see comments]. *BMJ* **298**: 707-710.
- Klaes R., Benner A., Friedrich T., Ridder R., Herrington S., Jenkins D., Kurman R.J., Schmidt D., Stoler M., & von Knebel D.M. (2002). p16INK4A immunohistochemistry improves interobserver agreement in the diagnosis of cervical intraepithelial neoplasia. *Am. J. Surg. Pathol.* **26**: 1389-1399.

- Klaes R., Friedrich T., Spitkovsky D., Ridder R., Rudy W., Petry U., Dallenbach-Hellweg G., Schmidt D., & von Knebel D.M. (2001). Overexpression of p16(INK4A) as a specific marker for dysplastic and neoplastic epithelial cells of the cervix uteri. *Int. J. Cancer* **92**: 276-284.
- Kobak W.H., Roman L.D., Felix J.C., Muderspach L.I., Schlaerth J.B., & Morrow C.P. (1995). The role of endocervical curettage at cervical conization for high-grade dysplasia. *Obstet. Gynecol.* **85**: 197-201.
- Melnikow J., Nuovo J., Willan A.R., Chan B.K.S., Howell L.P. (1998). Natural history of cervical squamous intraepithelial lesions: a meta-analysis. *Obstet. Gynecol.* **92**:727-35.
- Mitchell M.F., Tortolero-Luna G., Wright T., Sarkar A., Richards-Kortum R., Hong W.K., Schottenfeld D. (1996). Cervical human papillomavirus infection and intraepithelial neoplasia: a review. *J Natl Cancer Inst Monog* **21**:17-25.
- Mogensen S.T., Bak M., Dueholm M., Frost L., Knoblauch N.O., Praest J., & Svanholm H. (1997). Cytobrush and endocervical curettage in the diagnosis of dysplasia and malignancy of the uterine cervix. *Acta Obstet Gynecol Scand* **76**: 69-73.
- National Health Service Cervical Screening Programme (1999). Histopathology Reporting in Cervical Screening. Working Party of the Royal College of Pathologists and the NHS Cervical Screening Programme. *NHSCSP publication* **10**, 16 pp. Sheffield, NHS Cancer Screening Programmes. Available at: www.cancerscreening.nhs.uk
- Obermair A., Wanner C., Bilgi S., Speiser P., Reisenberger K., Kaider A., Kainz C., Leodolter S., Breitenecker G., & Gitsch G. (1998). The influence of vascular space involvement on the prognosis of patients with stage IB cervical carcinoma: correlation of results from hematoxylin and eosin staining with results from immunostaining for factor VIII-related antigen. *Cancer* **82**: 689-696.
- Ostor A.G. (1993). Natural history of cervical intraepithelial neoplasia: a critical review. *Int J Gynecol Pathol* **12**:186-92
- Pathology and Genetics of Tumours of the Breast and Female Genital Organs (2003). World Health Organization Classification of Tumours, IARCPress, Lyon
- Pretorius R.G., Zhang W.H., Belinson J.L., Huang M.N., Wu L.Y., Zhang X., & Qiao Y.L. (2004). Colposcopically directed biopsy, random cervical biopsy, and endocervical curettage in the diagnosis of cervical intraepithelial neoplasia II or worse. *Am. J. Obstet. Gynecol.* **191**: 430-434.
- Robboy S.J., Kraus F.T. & Kurman R.J. (1994). Gross discription: processing and reporting of gynecologic and obstretic specimens. In: *Blaustein's pathology of the female genital tract.* (ed Kurman R.J.), 4 edn, pp. 1225-1240. Springer-Verlag.
- Robboy S.J., Russell P., Anderson M.C., & Bentley R.C. (2002). Cutup - the gross description, processing and reporting of specimens. *Pathology of the Female Reproductive Tract* 861-877.
- Singer A. & Monaghan J.M. (2000). Lower Genital Tract Precancer: Colposcopy, Pathology and Treatment., 2nd edn, Blackwell Science Ltd, Oxford.
- Smela M., Chosia M., & Domagala W. (1996). Proliferation cell nuclear antigen (PCNA) expression in cervical intraepithelial neoplasia (CIN). An immunohistochemical study. *Pol J Pathol* **47**: 171-174.
- Sobin L.H., Wittekind Ch. (eds.) (2002). TNM Classification of Malignant Tumours. 6th edition, Wiley-Liss, Inc. and WHO 2003.

Stoler M.H. & Schiffman M.A. (2001). Interobserver reproducibility of cervical cytologic and histologic interpretations. *JAMA* **285**: 1500-1505.

Vutuc C., Haidinger G., Waldhoer T., Ahmad F., & Breitenecker G. (1999). Prevalence of self-reported cervical cancer screening and impact on cervical cancer mortality in Austria. *Wien. Klin. Wochenschr.* **111**: 354-359.

6

Management of abnormal cervical cytology

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6.1 Executive summary

A woman with a high-grade cytological lesion, a repeated low-grade lesion or with an equivocal cytology result and a positive HPV test should be referred for colposcopy. The role of the colposcopist is to identify the source of the abnormal cells and to make an informed decision as to whether or not any treatment is required. If a patient requires treatment the colposcopist will decide on which is the most appropriate method of treatment for each individual woman. The colposcopist must also organise appropriate follow-up for each woman seen. Guidelines are given for the management of ASC-US and HSIL. LSIL is more difficult because currently there is no evidence to support any method of management as being optimal; repeat cytology and colposcopy is an option but HPV testing as an initial management option is not sufficiently selective.

Quality assurance and data collection of patient management are an important part of the management and follow-up of women referred with an abnormal cervical smear.

All of these issues are addressed in this chapter.

6.2 Introduction

An abnormal Pap smear result indicates the possible presence of a progressive neoplastic lesion, which without treatment might evolve to a life-threatening cancer. Nevertheless, as already described in Chapter 2, a mild lesion is very likely to regress spontaneously, especially in young women, and therefore does not necessarily need treatment. The cytological suspicion of a high-grade lesion incurs a considerable risk of underlying severe dysplasia, which has a high chance of progression to cancer. These women should always be referred for colposcopy and biopsy. Appropriate treatment and/or follow-up must be offered based on the cytological, colposcopic and histological results and taking the particular clinical situation into account.

This chapter starts with a description of the procedures used when a smear is abnormal; i.e. repeat Pap smear, HPV testing, colposcopy, colposcopically directed punch biopsies or excision of the transformation zone. Endo-cervical evaluation by cytology or curettage is sometimes used when colposcopy is unsatisfactory or when an endo-cervical lesion is suspected. These diagnostic procedures are described in section 3. Histological examination of tissue material has already been described, in more detail, in the previous chapter. A special subsection (3.3) deals with the technique, the interpretation and the terminology of colposcopy.

Section 4 discusses therapeutic procedures currently used in Europe.

In sections 5 and 6, the procedures for each cytological category of the Bethesda 2001 classification and for histologically confirmed CIN are described. Recommendations are based on current knowledge of the natural history of lesions and available evidence concerning the accuracy of diagnostic procedures and efficacy of therapeutic interventions.

Complications following treatment can and do occur (see section 7). The three options to monitor the outcome after treatment (repeat cytology, HPV testing or colposcopy) are presented in section 8.

Finally recommendations for particular clinical situations are provided; e.g. pregnancy, immuno-suppression, HIV infection, post-menopause, adolescence and cyto-colpo-histological disparity. The chapter ends with recommendations for quality assurance in patient management and some general advice on how to communicate screening, diagnosis and treatment results to the woman concerned. Finally, a data collection format is attached.

6.3 Diagnostic evaluation of the abnormal smear

6.3.1 Repeat cytology

The cervical epithelium needs time to regenerate after cytology. Repeat cytology should not be performed less than 3 months after a previous Pap smear. Repeating the Pap smear is an acceptable option when a smear reports ASCUS/ASC-US¹ or LSIL or is unsatisfactory. In the latter case, it is useful for the laboratory to provide advice and sampling devices to the smear taker (see Chapter 3, appendix 1). Anti-microbial treatment is indicated before re-sampling if there is any suspicion of infection. Similarly, if the first smear was atrophic a second smear is recommended after topical oestrogenic treatment. The test performance of repeat cytology in case of atypical squamous cells of undetermined significance or LSIL is addressed in sections 6.5.1 and 6.5.2.

6.3.2 HPV testing

Recently, HPV DNA testing has been proposed as an alternative management option for women with minor cytological lesions, allowing the clinician to select women needing colposcopic and histological assessment (Wright *et al.*, 1995; Arbyn *et al.*, 2004a; Arbyn *et al.*, 2004b). When liquid-based cytology is used, a reflex HPV DNA test can be performed using the residual liquid from women with an ASCUS result without the necessity to recall the woman. Nevertheless, HPV reflex testing can also be performed on a separately submitted specimen taken with brush.

¹ In this chapter both "ASCUS" and "ASC-US" are used. Recommendations on management in the current guideline distinguish ASC-US (atypical squamous cells of undetermined significance) from ASC-H (atypical squamous cells where HSIL cannot be excluded) according to the 2001 version of the Bethesda System for cervical cytology (Solomon *et al.*, 2002 and Annex 2 of Chapter 3). However, most literature references addressing the natural history or management of atypical or borderline squamous lesions use the more general term "ASCUS", as defined in the 1988 or 1991 versions of The Bethesda System. The term ASCUS is accordingly maintained in this chapter when referring to literature in which the same term is used. ASCUS comprises ASC-US, ASC-H and also atypical cells suggesting a reactive process (ASC-R). This last subcategory is downgraded in TBS-2001 to "negative for intra-epithelial lesion of malignancy". ASCUS is maintained as a term in this chapter when referring to literature where this term is used.

6.3.3 Colposcopy

In the context of a woman with an abnormal smear, the aims of colposcopy are:

1. to determine the precise geographical/anatomical position of the TZ
2. to confirm or refute the cytological suspicion of CIN
3. to recognize or rule out invasive cancer
4. to recognize or rule out glandular disease
5. to facilitate treatment of and monitor progression or regression of CIN

The colposcope was described first by Hinselmann (Hinselmann, 1925). The modern colposcope is more sophisticated than that described by Hinselmann but its basic principle remains the same, namely that it allows the cervix to be viewed at magnifications between 6X and 40X. Colposcopy is used for three purposes:

1. To assess women with abnormal cervical cytology
2. To assess women with a clinically suspicious cervix
3. As a basic screening tool at the time of gynaecological examinations: this is how it was used by Hinselmann and it is still used in this way in some countries in Europe and Latin-America, usually in conjunction with cervical cytology. Colposcopy used in this way has a relatively high sensitivity for detecting pre-malignant disease but its specificity is too low for the purpose of population screening.

6.3.3.1 The transformation zone

The transformation zone is that part of the cervix which in foetal life was covered by columnar epithelium but which by process of metaplasia becomes squamous. This is a normal phenomenon that occurs in every woman. The area of columnar epithelium which is transformed to squamous by the process of metaplasia is referred to as the transformation zone. The stimulus to the process of metaplasia is vaginal pH. Under the stimulus of maternal oestrogen prior to birth and then shortly after birth the process of metaplasia begins. It is then arrested until the woman reaches puberty at which time under the stimulus of her own oestrogen, the vaginal pH again becomes acid and any columnar epithelium exposed to the vaginal acidity is transformed by metaplasia to new squamous epithelium. The importance of the transformation zone is that it is here that CIN develops, which if not detected and removed may progress to invasive squamous carcinoma.

The transformation zone is easy for the colposcopist to identify due to the presence of Nabothian cysts or follicles, gland openings, and typical branching vessels.

6.3.3.2 Technique of colposcopy

After due counselling, the woman adopts the modified lithotomy position. After macroscopic (naked eye) examination of the vulva, a vaginal speculum is inserted to allow exposure of the cervix. The size of the speculum used will depend on the anatomy of the vagina. The cervix is washed with normal saline, thereby removing any excess mucus, blood or vaginal discharge. At this stage the use of a green filter will enhance the examination of the capillary angioarchitecture (Jordan, 1985; Sellors & Sankaranarayanan, 2003).

A 3 or 5% solution of acetic acid is then applied to the cervix following which any premalignant disease should appear "aceto-white." The degree of aceto-whiteness should be assessed after a minimum period of 20 seconds. Acetic acid causes tissue oedema and superficial coagulation of intra-

cellular proteins, thereby reducing the transparency of the epithelium. When this happens the sub-epithelial capillaries are less easily visible and the epithelium itself appears white. The reason that colposcopy has a low specificity is that not all aceto-white epithelium is premalignant.

Aceto-white epithelium can be observed in the following situations:

1. Immature squamous metaplasia
2. Healing or regenerating epithelium
3. Congenital transformation zone
4. Human papilloma virus infection
5. Cervical intraepithelial neoplasia
6. A combination of CIN and HPV
7. Glandular intraepithelial neoplasia
8. Invasive squamous cell carcinoma
9. Adenocarcinoma

The colposcopist is taught to recognise original squamous epithelium, columnar epithelium, the squamo-columnar junctions and the transformation zone. It is in the transformation zone that premalignant changes are found and so it is important for the colposcopist to identify, recognise and assess the transformation zone and decide whether the transformation zone is normal or abnormal. The congenital transformation zone (CTZ) is that part of the cervix (and sometimes the vagina) which in foetal life was columnar epithelium but which during foetal life and immediately after birth becomes transformed from columnar epithelium to squamous epithelium by the process of metaplasia. The CTZ is sometimes difficult to recognise but its characteristic features are that it is faintly aceto-white, is non-staining with iodine, and to the unwary eye can be confused with low-grade CIN or VAIN.

If the squamo-columnar junction cannot be seen because the transformation extends into the endo-cervical canal, then an endo-cervical speculum should be inserted into the lower part of the endo-cervical canal to allow inspection. If the transformation zone can be seen in its entirety the colposcopy is graded satisfactory. If, on the other hand, the transformation zone cannot be seen in its entirety (because the SCJ extends into the endo-cervical canal beyond the reach of the colposcope) then the colposcopy is deemed unsatisfactory.

The application of Lugol's iodine (Schiller's test) causes a homogeneous dark brown staining of normal squamous epithelium. The principle behind this is that normal squamous epithelium is rich in glycogen which stains brown with iodine. On the other hand premalignant cells are deficient in glycogen and, therefore, are relatively non-staining. Iodine uptake gradation has been used for demarcating abnormal areas prior to treatment. However, most experienced colposcopists do not find any great benefit from the routine use of iodine. It should be remembered that not all non-glycogenated epithelium is abnormal: immature squamous metaplasia, healing/regenerating epithelium, congenital transformation zone and normal epithelium affected by HPV may also be non-glycogenated and therefore non-staining with iodine.

NOTE: A Schiller-positive test is an area which is non-staining with iodine!

6.3.3.3 Colposcopic features suggestive of CIN

There are changes in the subepithelial angioarchitecture that are apparent in premalignant disease.

These can be summarised as follows:

1. Punctation: either fine or coarse depending on the severity of the lesion
2. Mosaic: either fine or coarse depending on the severity of the lesion
3. Atypical vessels: suggestive of associated carcinoma
4. The degree of aceto-whiteness: high-grade lesions are more densely aceto-white than low-grade lesions.
5. Borders of the lesion: low-grade lesions have feathery indistinct or finely scalloped edges, while high-grade lesions have sharp straight edges.

The colposcopic features of a low-grade lesion are: the lesion is faintly aceto-white. There may be no subepithelial vessels visible but if the vessels are visible they take the form of a fine punctation or mosaic. The lesions are non-staining with iodine.

The colposcopic features of a high-grade lesion are: dense aceto-white changes, not staining with iodine, moderate or coarse punctation or mosaic, presence of atypical vessels. If atypical vessels are very prominent and irregular then the possibility of underlying malignancy should be considered.

There is huge overlap between normal and abnormal epithelium for each of these indices of abnormality.

6.3.3.4 Colposcopic terminology

The International Federation for Cervical Pathology and Colposcopy (IFCPC) approved a revised colposcopic classification and basic colposcopic terminology in 2002 (see Table 1). As the primary organisation of colposcopists and cervical cytologists, IFCPC recommended that this updated format be used for clinical diagnosis, treatment and research in cervical cancer (Walker *et al.*, 2003).

Table 1. International Federation for Cervical Pathology and Colposcopy (IFCPC) classification for colposcopy²

I.	Normal colposcopic findings
	Original squamous epithelium
	Columnar epithelium
	Transformation zone
II.	Abnormal colposcopic findings
	Flat acetowhite epithelium
	Dense acetowhite epithelium*
	Fine mosaic
	Coarse mosaic*
	Fine punctation
	Coarse punctation*
	Iodine partial positivity
	Iodine negativity*
	Atypical vessels*
III.	Colposcopic features suggestive of invasive cancer
IV.	Unsatisfactory colposcopy
	Squamocolumnar junction not visible
	Severe inflammation, severe atrophy, trauma
	Cervix not visible
V.	Miscellaneous findings
	Condylomata
	Keratosis
	Erosion
	Inflammation
	Atrophy
	Deciduousis
	Polyps

* indicates the characteristics of high-grade changes (dense acetowhite epithelium, coarse mosaic, coarse punctation, thick leukoplakia, atypical vessels); characteristics of low-grade changes are faint acetowhite epithelium, fine mosaic, fine punctuation, thin leukoplakia.

² Reproduced with permission of The American College of Obstetricians and Gynaecologists (Walker *et al.*, 2003).

6.3.3.5 The new transformation zone classification

One of the most important recommendations in the new IFCCPC classification was to define three types of transformation zone (Walker *et al.*, 2003; Prendiville *et al.*, 2003). The system has three indices by which the transformation zone may be classified. These are:

1. the size of the ectocervical component of the transformation zone;
2. the position of the upper limit of the transformation zone; and
3. the visibility of the upper limit of the transformation zone.

The three types of transformation can be characterized as being completely ectocervical, fully visible with an endo-cervical component, or not fully visible (Fig 1). The qualification large or small refers to the ectocervical component of the transformation zone. Large means that the transformation zone occupies more than half of the ectocervical epithelium.

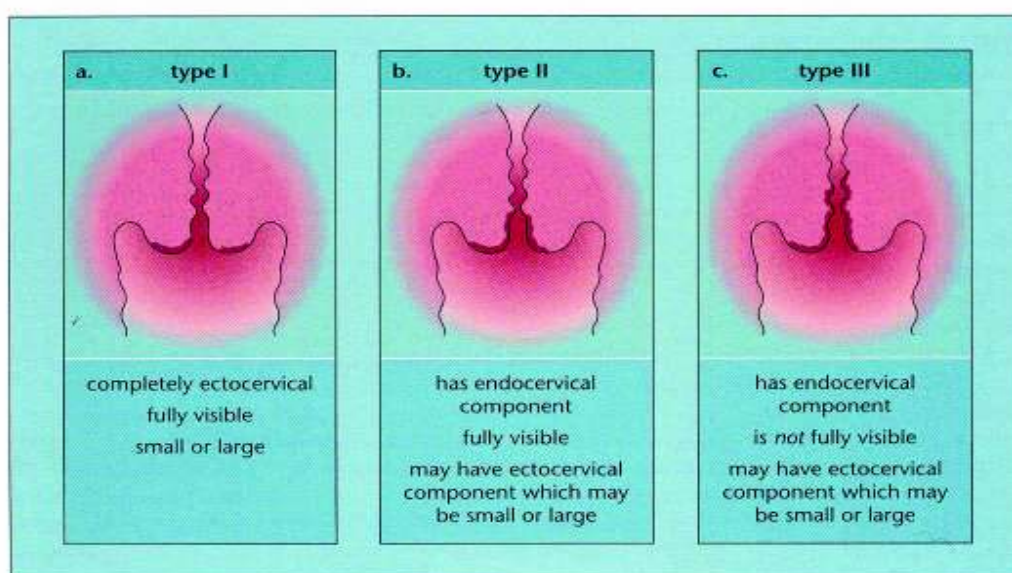


Fig. 1. The three types of transformation zone, as proposed by the new IFCCPC classification³

Table 2. Transformation zone geographical classification (Prendiville, 2003b)

Type of TZ	Size	Site	Visibility	Adequacy colposcopy
Type1	Small	Completely ectocervical	Fully visible	Satisfactory
Type1	Large	Completely ectocervical	Fully visible	Satisfactory
Type 2	Small	Partially endocervical	Fully visible	Satisfactory
Type 2	Large	Partially endocervical	Fully visible	Satisfactory
Type 3	-	Totally endocervical	Not fully-visible	Unsatisfactory
Type 3	Small	Partially endocervical	Not fully visible	Unsatisfactory
Type 3	Large	Partially endocervical	Not fully visible	Unsatisfactory

³ Fig. reprinted from Prendiville W. (2003). *The treatment of grade 3 cervical intraepithelial neoplasia. In: Colposcopy: management options* (eds Prendiville W., Ritter J., Tatti S. & Twiggs L.B.), pp. 129-133 with permission from Elsevier

These three different transformation zone types warrant an individualized therapeutic approach. Even if one uses an excisional technique for every circumstance, it is still necessary to modify the approach according to the type of transformation zone. If one utilizes LLETZ as the routine treatment modality, the shape and size of the loop needs to be modified according to the transformation zone type.

TZ type 1

It is entirely appropriate to use either an excisional or destructive method, provided the standard criteria are met, in order to successfully treat type 1 transformation zone. For a small TZ a loop of 2 x 1.5 cm can be used, whereas for larger TZ a wider loop or a combination of loops should be chosen.

TZ type 2

For a type 2 transformation zone it may be possible to use a destructive method but we would advocate an excisional procedure: a 2 x 2 cm or larger loop excision if the TZ is small, a combination of loops if the TZ is large.

TZ type 3

An excisional technique is mandatory for any type 3 TZ. The type 3 transformation zone has a high risk of incomplete excision. In this circumstance it may be wise to consider alternatives to LLETZ. Straight wire excision is such an alternative, as are cold knife or laser excision (Mor-Yosef *et al.*, 1990).

6.3.3.6 Diagnostic accuracy of colposcopy

Unbiased assessment of the sensitivity and the specificity of a test require the independent verification with a gold standard, which usually relies on histology. This is particularly difficult for colposcopy since the choice of the biopsy site depends on colposcopy itself. Because of this intrinsic dependency, accuracy estimates for colposcopy are inflated. Colposcopically negative cases are very often considered as truly negative without histological confirmation. Moreover, in case of glandular cervical lesions or endo-cervical location of the SCJ, colposcopy may be negative, even when intraepithelial neoplasia is present. In some cases the CIN can also be located in the gland clefts and may show a thin white rim around the gland opening (sometimes referred to as reverse mosaic or umbilicated mosaic).

In a meta-analysis conducted by Mitchell (1998), based on 9 studies, the sensitivity and specificity of colposcopy in detecting CIN2+, was estimated to be 96% and 48%. However, most studies included in the meta-analysis suffered from this bias. In one particular study conducted in Xanxi, China (Pretorius *et al.*, 2001; Pretorius *et al.*, 2004) a more unbiased assessment of colposcopic accuracy was revealed. Biopsies were taken not only from colposcopically suspect areas but also from the four quadrants of the transformation zone in colposcopically negative cases. Also, endo-cervical curettage was performed in every woman. In this study the sensitivity of colposcopy-directed biopsy for CIN2+ in women with satisfactory colposcopy was 57% (95% CI: 52-62%) (Pretorius *et al.*, 2004).

6.3.3.7 Colposcopic examination of the vagina and vulva

First, the vulva should be examined macroscopically. Inspection of the vaginal walls is part of a colposcopic examination. At completion of the colposcopic assessment of the cervix, the speculum should be withdrawn when the vaginal wall is being inspected. Any abnormalities may be identified and dealt with appropriately. If the cervical or vaginal surfaces look abnormal, then the vulva should be inspected colposcopically.

6.3.3.8 Colposcopy of the post-menopausal cervix

The postmenopausal cervix in women not using oestrogen replacement therapy may be atrophic. The cervical and vaginal epithelium becomes very thin, thereby allowing visualisation of the subepithelial capillaries, which in turn may appear red and atypical. The use of acetic acid is not as effective in detecting premalignant disease in these cases. If there is any difficulty in assessing the post-menopausal cervix, it is helpful to give a short course (3-4 weeks) of intravaginal oestrogen cream.

6.3.3.9 Colposcopy in pregnancy and in the post partum period

The indication for colposcopy in pregnancy is an abnormal cervical smear. Again after due counselling, the colposcopic assessment proceeds in the same way as in the non-pregnant woman. Colposcopic assessment of the pregnant cervix is more difficult than in a non-pregnant cervix because the cervix is larger, oedematous and more vascular. An ordinary cervical speculum may make access to the cervix difficult, in which case a large speculum should be used. The cervix is usually covered by mucus, which is difficult to remove, and in the primiparous patient in particular there may be immature metaplasia, which can confuse the issue. Decidual changes of the cervical epithelium can mimic cancerous epithelium. In addition to all of these factors, the vascular changes associated with abnormality may be more pronounced leaving the inexperienced colposcopist to conclude that the severity of the lesion may be more than it actually is.

A pregnant woman with an abnormal cervical smear should be assessed by an experienced colposcopist. If the colposcopist feels that there is any cytologic or colposcopic suggestion of malignancy then a colposcopically directed biopsy or biopsies should be taken, but in the absence of these features biopsy should be postponed until after pregnancy. Biopsies taken in pregnancy are often accompanied by bleeding and the sample itself is often unsuitable for good histological assessment. The cervix should be assessed with cytology and colposcopy at intervals of 3 months during the pregnancy, with final assessment being undertaken 3-4 months after delivery.

In the immediate puerperium, the cervix may also be difficult to assess. Prior to the first postnatal ovulation, particularly in the woman who is still breastfeeding, the cervix may appear atrophic which makes both cytology and colposcopy much more difficult. If this proves a problem for diagnosis, then a short course of vaginal oestrogen will be helpful. When there is no suspicion of malignancy, it is often prudent to wait until the oestrogenic state has returned to normal before undertaking colposcopy and/or treatment.

6.3.3.10 Conclusions for colposcopy

1. Colposcopy allows identification, localisation and delineation of premalignant lesions of the cervix, vagina and vulva and directs the biopsy site.
2. In some countries, colposcopy is used as a screening tool but because of its low specificity, it should not be used in primary screening but reserved for those women who have been shown to have abnormal cervical cytology.
3. Colposcopy must be performed prior to treatment of cervical intraepithelial neoplasia.
4. Colposcopy should be performed only by trained and experienced colposcopists (NHSCSP, 1996a; NHSCSP, 1996b; NHSCSP, 1997; NHSCSP, 2004a).
5. Colposcopists should audit their work to confirm that the outcome of their colposcopic assessment and colposcopically-directed treatment is in keeping with internationally agreed standards.

6. The colposcopic findings should be recorded in the patient's record.

6.3.4 Cervical biopsy

A cervical biopsy is taken under colposcopic vision from the areas that reveal the highest degree of suspected abnormality.

A small sample can be taken with one of several specially designed cervical biopsy forceps. The colposcopist should ensure that the best possible sample is given to the pathologist. The biopsy must include both the surface epithelium and the underlying stroma in order to decide whether the lesion is strictly intraepithelial or if it extends to the stroma. The biopsy must include interpretable material, it must show no signs of thermocoagulation and it must be fixed rapidly. A punch biopsy will often not be large enough to achieve sufficient depth whereby microinvasive disease can confidently be ruled out. Usually a local anaesthetic is not required although there is evidence that local analgesia is effective in reducing discomfort associated with punch biopsies (Martin & Prendiville, 2004). If necessary, more than one biopsy can be taken. A further technique for taking a small biopsy is to use a small diathermy loop, in which case local anaesthetic should be injected before taking the biopsy: this technique has the advantage of giving a good specimen with an adequate amount of stroma and without distorting tissue (Cartier & Cartier, 1993; Abdel-Hady *et al.*, 2001). These biopsies are superior to punch biopsies for revealing or ruling out microinvasive cancer (Prendiville *et al.*, 1986).

If bleeding occurs following the removal of the biopsy then it can be arrested using either diathermy or simply applying Monsel's solution (see annex 1) (Anderson *et al.*, 1996).

If endo-cervical material is required for histological examination, the colposcopist should take this using an endo-cervical curette or an endo-cervical brush (see 6.3.5).

The diagnostic quality of the histological examination of a biopsy may suffer from a number of imperfections. On the one hand, sampling error is a major cause for underreporting of lesions; on the other hand subjectivity of histological interpretation adds to the limitations in reliability (Ismail *et al.*, 1989; Stoler & Schiffman, 2001). For more precise instructions on biopsy taking, storage, transport, processing and examination, see Chapter 5.

6.3.5 Endo-cervical curettage

Endo-cervical curettage (ECC) aims to detect an endo-cervical squamous or glandular lesion that cannot be reached by a colposcopically directed biopsy.

Presence or absence of an invasive lesion cannot be confirmed because the specimen is often superficial. Moreover, ECC distorts the local architecture, compromising the distinction between adenocarcinoma in situ and invasive adenocarcinoma. Endo-cervical sampling using an endo-cervical brush shows a lower false-negative rate than ECC (Andersen *et al.*, 1988; Weitzman *et al.*, 1988; Hoffman *et al.*, 1993; Mogensen *et al.*, 1997).

In the US, ECC often is carried out in conjunction with cervical biopsy. It is used less frequently in Europe where more often a diagnostic conisation is preferred when an endo-cervical lesion has to be excluded (Gath *et al.*, 1995). Endo-cervical curettage should not be performed during pregnancy (Wright *et al.*, 2002).

6.4 Treatment procedures

The management of colposcopically confirmed disease can be ablative, excisional or in some circumstances observational. There is no obviously superior conservative surgical technique for treating and eradicating cervical intra-epithelial neoplasia (Martin-Hirsch *et al.*, 2000). This is true if success/failure rates are the index of superiority. Excisional techniques are preferred in the majority of circumstances because of their clear superiority over ablation in terms of histological evaluation of the transformation zone. Histological examination of the excised tissue allows the pathologist to recognize or rule out microinvasive cancer, glandular disease, margin involvement and depth of excision. It also allows the colposcopist to self-audit his/her diagnostic skills.

6.4.1 Excision of the lesion

The aim of an excisional treatment is to remove the lesion in its entirety. The entire excised specimen is then submitted for histological assessment. The sample can only be planned safely by colposcopic assessment of the lesion by an experienced colposcopist.

Excision of the TZ should not be performed for CIN 1, unless the lesion has persisted over a period of more than a year. It should be performed without delay in the presence of high-grade intra-epithelial neoplasia or suspicion of early stromal invasion or microinvasion.

Techniques used for the complete excision of the TZ are LLETZ, cold knife conisation, laser excision and NETZ. **Large loop excision of transformation zone (LLETZ)** consists of the excision of cervical tissue using a diathermy loop. LEEP (loop electrosurgical excision procedure) is a North American term used to describe the same technique as LLETZ. The terms LLETZ and LEEP are used synonymously, but in this guideline only the European term **LLETZ** will be used. In **cold knife conisation** cervical tissue is removed using a knife, and the excised product has the shape of a cone. **Laser excisional conisation** or **laser excision** means that cervical tissue is removed using a CO₂ laser in cutting mode. **NETZ** (needle excision of the transformation zone) means that the TZ is excised with a straight diathermy wire. **SWETZ** (straight wire excision of the transformation zone) and **NETZ** refer to the same technique.

When performing the excision the following recommendations should be followed:

1. The procedure should be carried out under colposcopic control.
2. The lesion together with the entire transformation zone should be removed.
3. It is helpful to mark the excised specimen with a thread at 12 o'clock, thereby facilitating the histopathologist to orient the specimen.
4. Surgeons should avoid damage of the ecto-cervical epithelium or of the endo-cervical canal.
5. A cervical dilator for orientation of the excision specimen is unhelpful.
6. The size and shape of the excised specimen will be determined by the colposcopic delineation of the lesion.
7. Excision should be mandatory if the lesion involves the endo-cervical canal.
8. If the lesion involves the endo-cervical canal, endo-cervical sampling should be considered after the excision.

9. Thorough histological assessment by a pathologist skilled in gynaecological pathology is essential.
10. The histopathologist should be informed of the cytology and colposcopic findings.
11. Cold knife conisation gives excision margins that are not affected by thermal artefact, whereas the margins of laser excisional cone or diathermy loop excision cone may be damaged. In skilled hands, the thermal artefact is generally minimal. In the meta-analysis of Martin-Hirsch *et al.*, (2000) there was a clear advantage of cold knife cone biopsy over laser or LLETZ.
12. Excision of the transformation zone in multiple fragments can complicate histopathological assessment. Furthermore, if microinvasive disease is present, it may be impossible to allocate a substage or define completeness of excision in fragmented excisional specimens. When using LLETZ, the external os and lower canal should be removed in a single sample. Disease lateral to the central area can be removed separately.
13. If cold knife conisation is performed great care must be taken to minimise side effects such as haemorrhage and cervical stenosis. Haemorrhage can be minimised by injecting the cervix pre-operatively with adrenalin 1 in 200,000. If haemorrhage is controlled with diathermy and the use of Monsel's solution (see annex 1, (Anderson *et al.*, 1996)) cervical stenosis is much less likely to occur than if cervical sutures are used to control bleeding at the time of conisation.

6.4.2 Local destructive therapy

The aim of local destructive therapy is to destroy CIN by the use of radical diathermy, laser vaporisation, cryotherapy or cold coagulation.

Radical diathermy (or electrocoagulation) uses a straight electrodiathermy needle and aims to destroy tissue to a depth of approximately 1 cm.

Diathermocoagulation is a technique which uses heat to destroy cervical epithelium only to a depth of 2-3 mm. The depth of destruction is too superficial for it to be recommended for the treatment of CIN.

Laser vaporisation employs a CO₂ laser at a high power setting: under colposcopic control the laser beam is aimed directly at the tissue to be removed: it works by vaporising the water in the cells at the speed of light.

Cryotherapy (or cryocautery) employs a probe which is applied directly to the tissue to be destroyed by freezing: the depth of destruction is 3-4 mm.

Cold coagulation uses a probe similar to a cryocautery probe, but destroys the tissue by heating it to 100°C.

All of these techniques can be performed on an outpatient basis. The dilemma is that the tissue is destroyed rather than being sent for histological assessment: the fear is that occasionally CGIN, AIS or early invasive carcinoma will remain undetected and, therefore, be treated inappropriately by destruction rather than by excision. This is one of the reasons why excisional techniques are preferred. However, provided that certain selection criteria are adhered to, the various techniques can be safe and very effective.

The selection criteria are as follows:

1. The entire transformation zone must be visible.
2. One or more biopsies should be taken from the area or areas that colposcopically show the most severe change.
3. The result of the biopsy or biopsies should be available prior to the destructive therapy.
4. Cryotherapy should not be offered to women with large lesions, occupying more than 75% of the ectocervix, extending to the vaginal wall or extending more than 2 mm beyond the cryoprobe (Gaffikin *et al.*, 2003; Denny *et al.*, 2005). This applies also to cold coagulation but not to radical diathermy.
5. There should be no evidence of invasive disease on cytology, colposcopy, or biopsy.
6. The Pap smear should not contain glandular atypical cells.
7. The destructive therapy should be carried out under colposcopic control by an experienced colposcopist.
8. There must be adequate follow-up.

When using an ablative therapy, destruction of the TZ should be to a minimum depth of 4mm (it is probably safer to aim to destroy to a depth of 7mm). Destruction should extend beyond the ectocervical and endo-cervical margins of the lesion (Anderson & Hartley, 1980; Boonstra *et al.*, 1990).

The evidence from an extensive systematic review of the literature is that cold coagulation and laser ablation are effective in treating all grades of CIN when used by skilled operators (Martin-Hirsch *et al.*, 2000). Radical diathermy can be very effective. Chanen & Rome reported a cure rate of 98.3% with a single treatment (Chanen & Rome, 1983). Cryocautery should only be used for type 1 transformation zones and a double freeze-thaw-freeze technique should be used (Schantz & Thormann, 1984).

Ablative therapy should aim to destroy the entire transformation zone as more localised treatment produces higher recurrence rates (Burke *et al.*, 1980).

6.5 Management of patients according to the severity of cytological abnormalities

In the next section, management procedures are proposed according to the type and the severity of the reported cytological abnormalities. Management of histologically confirmed CIN will be addressed in the next section. The decision to treat and the choice of treatment must be based on the natural history of the lesion (see Chapter 2), and the probability of cytological sampling and/or interpretation error (see Chapter 3).

The approaches chosen to manage cytological abnormalities should make allowances for individual characteristics, such as age, fertility status and likely attendance for follow-up, risk profile and immune status.

6.5.1 Management of women with atypical squamous cells of undetermined significance

6.5.1.1 Data providing evidence

Melnikow *et al.* (1998) reviewed data published between 1970 and 1996, and pooled regression and progression rates, separated by a period of follow-up for each category of TBS 1991, using meta-analytical methods. The probability of progression of ASCUS to invasive disease over 6 months and to HSIL over 24 months was 0.06% and 0.25%, respectively. In the Norwegian screening programme, the relative risk of CIN2+ within 2 years after an ASCUS diagnosis compared to women with a negative result was 15 to 30 (Nygard *et al.*, 2002). In the ALTS trial, the 24-month cumulative incidence of CIN3+ among women with an index smear showing ASCUS varied between 8 and 9% (ASCUS-LSIL Triage Study Group, 2003b). In a meta-analysis of the diagnostic performance of management methods for women with a prior ASCUS result, the pooled prevalent risk of CIN2+ was 10% and the risk of CIN3+ was 6% (Arbyn *et al.*, 2004a; Arbyn *et al.*, 2004b). These data indicate that women with ASCUS need further evaluation.

In the aforementioned meta-analysis of ASCUS triage, the pooled (cross-sectional) sensitivity of repeat cytology for the presence of histologically confirmed CIN2+, using ASCUS or worse as the positive triage result, was estimated to be 82% (95% CI: 78% to 84%), whereas the pooled specificity was only 58% (95% CI: 50-66%) (Arbyn *et al.*, 2004a). The sensitivity of a repeat smear using LSIL or HSIL as the cut off was substantially lower.

The pooled sensitivity and specificity for CIN2+ of the Hybrid Capture 2 (HC2) assay, was 95% (95% CI: 93-97%) and 67% (95% CI: 58-76%), respectively. The HC2 test positivity rate was 41% overall, and varied from 29 % (Morin *et al.*, 2001) to 88% (Solomon *et al.*, 2001). The sensitivity ratio (sensitivity of repeat cytology at the threshold of ASCUS+/ sensitivity of HC2) was 1.16 (95% CI: 1.04-1.29) indicating a sensitivity for HC2 being, on average, 16% higher than for repeat cytology. The specificity of HC2 was higher as well, but the difference was not significant (ratio of 1.05; 95% CI: 0.96-1.15). The relative accuracy of both triage strategies using CIN3+ as the outcome showed similar results as for CIN2+ (Arbyn *et al.*, 2004b).

In the ALTS study, the (longitudinal) sensitivity for a CIN3+ diagnosis within 2 years, using the HC2 assay at enrolment, was estimated at 92%. Fifty-three percent of women were HPV+ and were referred for colposcopy (ASCUS-LSIL Triage Study Group, 2003b). The longitudinal sensitivity of cytology repeated every 6 months for 2 years, using ASCUS as the cytology threshold was similar, but in this strategy, 73% of women required referral to colposcopy. Remarkably, immediate colposcopy, showed a lower sensitivity for cumulative CIN3 than HC2.

6.5.1.2 Management options in case of ASCUS

Three options can be considered when the presence of atypical squamous cells of undetermined significance is reported: hrHPV DNA testing, repetition of the smear, and referral for colposcopy. Reflex hrHPV DNA testing is the preferred option when liquid-based cytology is used and HPV tests are available (Wright *et al.*, 2002; Arbyn *et al.*, 2004a). HPV-positive cases should be referred for colposcopic evaluation. HPV testing can be repeated after 12 months (Cox *et al.*, 2003; Guido *et al.*, 2003; Cuzick *et al.*, 2003) when no CIN is found on colposcopy and biopsy. HPV-negative women should be recommended to have an additional Pap smear taken after 1 year (Wright *et al.*, 2002).

A second acceptable option is a repeat smear after 6 to 12 months. If it is negative then the woman can be referred back to a normal screening schedule. If the first repeat smear is again ASC-US then a repeat smear is recommended within the next 6 to 12 months and if the further repeat

smear is again ASC-US then the woman should be referred for colposcopy. If any of the follow-up smears is greater than ASC-US then referral for colposcopy is advised. National guidelines may vary slightly in this particular recommendation and, therefore, clinicians should be guided by their own National guidelines.

Referral for immediate colposcopy is another alternative, which many experts consider to be over-management (Coleman *et al.*, 1993; Arbyn *et al.*, 1996; Sawaya, 2005). It may be the preferred choice when poor follow-up compliance is suspected or when explicit risk factors are present. Immediate referral for colposcopy should be no more than a very low percentage of cases of ASC-US. If colposcopy does not show CIN, a repeat smear after 1 year is recommended.

For women with ASC-US who have clinical or cytological signs of atrophy, a repeat smear after a course of intra-vaginal estrogen is recommended. When ASCUS is accompanied with excessive inflammation due to an infection, appropriate anti-microbial treatment is indicated before repetition of the smear. Pregnant women with ASC-US should be managed as non-pregnant women.

6.5.1.3 Management of ASC-H

Women with atypical squamous cells, where the presence of HSIL is suspected (ASC-H), should be referred for colposcopy. When colposcopy is negative, and when the diagnosis of ASC-US is upheld after review of cytology, colposcopy and histology, a repeat smear at 6 and 12 months or hrHPV DNA test at 12 months is recommended (Wright *et al.*, 2002). Such cases should be discussed in a multi-disciplinary meeting.

6.5.2 Management of women with LSIL

6.5.2.1 Data providing evidence

The natural history of LSIL is reviewed in Chapter 2. Important elements for the management of women with LSIL are summarised below.

Melnikow's meta-analysis showed that the progression of low-grade lesions increased significantly by length of follow-up. For LSIL, the cumulative rate of progression to HSIL was 6.6% (95% CI: 1.1% to 12.1%) after 6 months and 20.8% (95% CI: 6.1% to 35.6%) after 24 months. Probably the best documented natural history of cervical dysplasia is the study of Holowaty *et al.*, who studied cohorts included in the Toronto cytological registry linked to the Ontario cancer registry (Holowaty *et al.*, 1999). It was estimated that within 24 months 44.3% (95% CI: 43.0% to 45.5%) of mild dysplasia regressed to normal; 0.6% (95% CI: 0.5% to 0.7%) progressed to CIN3 and 0.1% (95% CI: 0.0% to 0.1%) to cancer, whereas over 10 year 87.7% of women showing mild dysplasia (95% CI: 86.0% to 89.5%) became normal, 2.8% (95% CI: 2.5% to 3.1%) progressed to CIN3 and 0.4% (95% CI: 0.3% to 0.5%) to invasive cancer.

In a meta-analysis of studies examining triage of women with LSIL, the pooled sensitivity of repeat cytology was 92% (95% CI: 84%-98%) with a specificity of 42% (95% CI: 27%-56%) (Arbyn *et al.*, 2005; Arbyn *et al.*, 2006). The HC2 test showed a pooled sensitivity for CIN2+ of 95% (95% CI: 91-100%) and a specificity of only 33% (95% CI: 18-48%). The sensitivity and specificity ratios did not differ significantly from unity. Both triage methods showed low specificity. The hrHPV test positivity rate varied between 58% (Bergeron *et al.*, 2000) and 88% (Ferris *et al.*, 1998) and its pooled value was 77% (95% CI: 67-86%). On average, among women with LSIL, 17% (95% CI: 10-23%) have prevalent CIN2+ and 12% (95% CI: 5%-19%) have prevalent CIN3+.

Several longitudinal studies, spanning 1 to 3 years of follow-up, indicate increased progression and decreased regression rates as well as shorter progression and longer regression duration in hrHPV positive LSIL patients compared to HPV negative LSIL cases (Remmink *et al.*, 1995; Nobbenhuis *et al.*, 1999; Nobbenhuis *et al.*, 2001; Van Duin *et al.*, 2002; Schlecht *et al.*, 2003). In the ALTS trial, the 2-year cumulative incidence of CIN3 among women with LSIL varied between 14% and 18% (ASCUS-LSIL Triage Study Group, 2003a). One repeat Pap smear at cut-off ASCUS had a sensitivity of 91% and resulted in referral of 81% of women. Cytology repeated every 6 months over 2 years allowed detection of all cases of CIN3 but resulted in referral to colposcopy of 89% of women. One HC2 test for the detection of hrHPV types at enrolment showed a sensitivity of 95% and a referral rate of 85% (ASCUS-LSIL Triage Study Group, 2003a). An HC2 test 12 months after a first report of LSIL detected 92% of cumulative CIN3+ and was associated with a referral rate of 55% (Guido *et al.*, 2003).

Further research is needed to identify more specific tools to distinguish LSIL women who are truly at risk for progressive lesions. HPV DNA typing, type-specific viral load, targeting essentially HPV 16, presence of hrHPV RNA and other progression markers are potential candidates. Currently evidence does not support any method as being optimal.

6.5.2.2 Management options in case of LSIL

Two management options can be proposed for woman with low-grade squamous intraepithelial lesions: repetition of the smear and referral for colposcopy. In most settings, hrHPV testing as an initial management option is not sufficiently selective.

Repetition of the Pap smear is an acceptable strategy. Observation tends to be the preferred management particularly in young nulliparous women. The smear may be taken at 6-month intervals until two subsequent negative smears have been obtained, and referral for colposcopy is advised if one of the smears shows ASC-US or a more severe lesion. Potential loss to follow-up should be taken into account before choosing this option.

Given the higher prevalence of high-grade CIN in case of LSIL compared to ASC-US, referral to colposcopy can be chosen as the preferred option.

When colposcopy is satisfactory and shows no lesions, a repeat smear or hrHPV DNA testing 12 months later is useful.

The recommendations concerning the management of ASC-US cases in post-menopausal women and women with infection also apply if LSIL is present (NHSCSP, 2004a).

6.5.3 Management of women with HSIL

6.5.3.1 Data providing evidence

In Melnikow's meta-analysis, the probability of progression from HSIL to invasive cancer at 24 months was estimated to be 1.4% (0%, 4.0%) (Melnikow *et al.*, 1998). The probability of regression was 35%. Holowaty found a cumulative progression to cancer after 2 years of 0.3% and 1.6% in women with, respectively, moderate or severe dysplasia. The 10-year cumulative rates were 1.2% for moderate and 3.9% for severe dysplasia (Holowaty *et al.*, 1999).

The rate of hrHPV-positivity in HSIL is, in general, higher than 90%, and may even reach up to 100% depending on the HPV testing system used.

6.5.3.2 Management options in case of HSIL

Referral for colposcopy and biopsy is the rule when a Pap smear shows a high-grade squamous intra-epithelial lesion. Triage using repeat cytology or HPV DNA detection is not indicated. If colposcopy is satisfactory and colposcopy and biopsy rules out the presence of high-grade CIN, a review of cytology and histology is recommended (Wright, 2002). Management should be decided according to the reviewed diagnosis. If the cytological interpretation of HSIL is upheld, excision of the transformation zone is recommended providing the woman is not pregnant (Prendiville, 2003b). If colposcopy is un-satisfactory, presence of an endo-cervical localisation of the lesion must be ruled out, therefore diagnostic excision of the transformation zone or conisation should be performed.

The choice of treatment for women with HSIL will depend on the suspected diagnosis, the size and type of transformation zone, the risk of default to follow-up, age and fertility aspirations.

In a number of reporting schemes (for instance the Munich report scheme), smears suggestive of CIN2 (moderate dysplasia) are grouped with cells suggestive of CIN1 (German report scheme). In this situation, the management recommendations described in this section are restricted to a cytological report of severe dysplasia (changes suggestive of CIN3).

6.5.4 Management of women with glandular cytological abnormality

The cytology report should clearly define if the cytological glandular abnormality relates to cervical or endometrial glandular cells or indicate if the type of glandular cells can not be clearly identified (Solomon *et al.*, 2002).

6.5.4.1 Data providing evidence

The natural history of glandular cervical lesions and the accuracy of cytology for detection of glandular intraepithelial or invasive disease is poorly documented (Kurman *et al.*, 1994). Nevertheless, several studies indicate that the presence of atypical glandular cells (AGC) in Pap smears is associated with a high frequency of underlying high-grade (endo-) cervical neoplasia or cancer (Taylor *et al.*, 1993; Kennedy *et al.*, 1996; Eddy *et al.*, 1997; Duska *et al.*, 1998; Ronnett *et al.*, 1999; Soofer & Sidawy, 2000; Valdin *et al.*, 2001). The prevalence or short-term cumulative incidence of invasive disease (squamous, adenosquamous or endometrial cancer) varies from <1% to 8% in follow-up series of women with glandular Pap smear abnormalities. The predictive value is considerably higher when presence of AGC cells is reported than in women with ASCUS or LSIL. Therefore, women with glandular cytological abnormalities **require particularly careful evaluation**. Repeat cytology is insufficiently sensitive to detect CGIN or invasive adenocarcinoma compared to colposcopy and endo-cervical and endometrial explorative methods (Kim *et al.*, 1999). Insufficient data are available concerning the performance of HPV DNA testing. Age is an important predictor for the origin of a glandular lesion: younger women most often have endo-cervical lesions whereas endometrial carcinoma generally occurs in older women. The clinician should be aware that abnormal glandular cells may originate in the uterus, Fallopian tube or ovaries and may require appropriate assessment.

6.5.4.2 Management options in case of glandular lesions

Direct referral for colposcopic, endo-cervical and/or endometrial exploration is indicated when a cytological result of atypical glandular cells or endo-cervical adeno-carcinoma in situ (AIS) is reported. If a woman with AGC suggestive either of neoplasia or endo-cervical AIS has negative colposcopy, a diagnostic conisation should be carried out. Cold knife excision is recommended in order to avoid destruction of the margins. When the indication for referral is AGC not otherwise specified and colposcopy reveals no neoplasia, repeat cytology every 6 months for 2 years using additional endocervical brush sampling is recommended. Gynaecologic exploration should be offered if one of the follow-up smears shows any degree of squamous or glandular abnormality.

When the glandular lesion is qualified as being endometrial, and if the woman is older than 35 or if there is unexplained vaginal bleeding when the woman is younger than 35, endometrial sampling in addition to colposcopy is indicated to exclude endometrial carcinoma (Wright *et al.*, 2002).

6.5.5 Management of cervical smears showing endometrial cells

While cervical screening does not aim to detect endometrial carcinoma, occasionally the cervical smear will detect endometrial cells, with or without abnormality, and will contribute in some cases to the earlier diagnosis of endometrial carcinoma. For the cytopathologist there is the dilemma that a final interpretation of the findings often cannot be based on morphology alone, and so has to consider age, menstrual history, hormonal treatment (e.g. oestrogen replacement therapy) and the presence or absence of an intrauterine device. If the history is incomplete then the cytologist will need to address this problem in the report.

While for the assessment of cervical lesions (both squamous and glandular) repeat cytology, HPV testing and colposcopy are available and can be useful tools in deciding on further management, the options in the presence of abnormal endometrial cells are limited. In this scenario, the question is whether or not hysteroscopy and curettage of the endometrial cavity is indicated. Follow-up by repeat cervical cytology is not appropriate because the endometrial cells may be shedding intermittently.

Depending on the cytological aspect of the endometrial cells in the smear, the patient's age, the hormonal status and presence of IUD, the following management can be recommended:

1. Endometrial cells in keeping with the stage of the cycle: no need for further investigation.
2. Endometrial cells not in keeping with the stage of the cycle: no need for further investigation in young women but may require assessment in older women.
3. Endometrial cells in women with an IUD: no need for further investigation.
4. Normal appearing endometrial cells in a post menopausal woman: this would always warrant further assessment even if the woman is using oestrogen replacement therapy.

The minimum assessment should be a vaginal ultrasound to assess endometrial thickness: if this is 4 mm or less, no further assessment is required. If the thickness is more than 4 mm then the endometrium should be sampled either by an outpatient endometrial biopsy or preferably by endometrial biopsy or curettage or hysteroscopy and curettage.

5. Atypical endometrial cells or cytological findings suggestive of endometrial adenocarcinoma: the woman should be referred for ultrasound, hysteroscopy and biopsy or diagnostic curettage.

6.6 Management of histologically confirmed CIN

6.6.1 Management of CIN1

While some 60-70% of histologically suspected cases will revert to normal over time, some 15% will persist. Between 0% and 30% will ultimately reveal CIN2-3 and less than 1% will lead to invasive carcinoma (Soutter *et al.*, 1986; Bolger & Lewis, 1988; Anderson *et al.*, 1992). However, colposcopists have to be aware that the diagnosis of CIN1 is not always reliable. This is illustrated by the wide range of intra-observer and inter-observer variability in the diagnosis of colposcopically directed biopsies initially classified as CIN1, as demonstrated in the ASCUS/LSIL Triage Study (Stoler, 2001 11682 /id). In this study only 43% were confirmed as having CIN1 by expert panel review, 41% were downgraded to normal and 13% were upgraded to CIN2 and 3. Further evidence for the potential unreliability of colposcopic biopsies suggesting CIN1 is illustrated by studies that compared subsequent loop excisions of the transformation zone. These studies have demonstrated CIN2 and 3 in 23-55% of specimens (Massad *et al.*, 1996). The management of low-grade disease has to balance the high chance of spontaneous regression and negative histology with the possible risk of not treating underreported or missed high-grade disease. Observational and immediate treatments both have advantages and disadvantages.

Two different situations can be distinguished: satisfactory and unsatisfactory colposcopy.

Satisfactory colposcopy

Two options can be recommended: follow-up or treatment. Follow-up consists of repeat cytology at 12 and 24 months or hrHPV DNA testing at 12 months, with referral for colposcopy when cytology reports ASC-US or a more serious lesion or when the HPV test is positive. Observation tends to be the preferred management, particularly in young nulliparous women (Moscicki *et al.*, 2004). There is no reliable evidence on the optimal duration of follow-up or whether colposcopy increases the detection of high-grade disease during this period.

Patients with CIN1 can also be offered treatment, which can be ablative or excisional. In case of recurrent CIN1 excisional methods should be preferred.

Unsatisfactory colposcopy

If colposcopy is unsatisfactory then an excisional treatment, should be considered, because occult high-grade disease might be present (Spitzer *et al.*, 1998).

Unacceptable treatment approaches for CIN 1:

1. See and treat: this refers to seeing a patient for the first time in the colposcopy clinic and removing the transformation zone by loop excision because the cervical epithelium shows aceto-white changes. For low-grade cytological abnormality this will result in a very large number of women receiving unnecessary treatment.
2. Local destruction procedures are unacceptable for CIN1 in patients with an unsatisfactory colposcopic examination (Spitzer *et al.*, 1998).
3. Podophyllin or podophyllin-related products are unacceptable for use in the vagina or on the cervix.
4. Hysterectomy as the primary and principle treatment for biopsy-confirmed CIN 1 is unacceptable unless there is another indication for hysterectomy such as a fibroid uterus.

6.6.2 Management of CIN2 and CIN3

The natural history of histologically confirmed high-grade CIN is documented only from a few small case-series, since these lesions are almost always treated. The review of Ostör (1993) included six studies, showing the outcome of 423 women with biopsy-proven CIN2 or CIN3 (Galvin *et al.*, 1955; Peckham & Greene, 1957; Lange, 1960; Lambert & Woodruff, 1963; Pahl *et al.*, 1965; Fu *et al.*, 1981). The pooled progression rate to carcinoma in situ or cancer was 20%, but varied widely (from 0% to 53%). The overall persistence rate was 50% (ranging from 15% to 96%) and the overall regression rate was 29% (ranging from 4% to 67%).

Women with high-grade CIN require treatment; observational follow-up is not an option. Local ablation or destruction, using laser ablation, cryotherapy, cold coagulation or radical diathermy is acceptable management strategies if colposcopy is satisfactory. In the case of recurrence or when colposcopy is unsatisfactory, excision using LLETZ or cold knife must be chosen (Wright *et al.*, 2003; Prendiville, 2003a).

Of these two approaches ablation or excision, excision is preferred. If destructive or ablative therapy is offered then the conditions outlined earlier must be adhered to.

6.6.3 Micro-invasive cancer

If the degree of invasion is no more than early stromal invasion, then local excision is adequate treatment.

If the lesion is microinvasive squamous carcinoma (FIGO Stage 1A1), it is still appropriate to use conservative excisional techniques alone, providing that the following conditions prevail (Wright *et al.*, 2003):

1. The excision margins are free of CIN and invasive disease.
2. The pathologist plus the multidisciplinary team have reviewed the histology and confirmed that the lesion is no more advanced than Stage 1A1.
3. If the invasive lesion has been excised but CIN extends to the excision margin (ectocervical and/or endo-cervical), then a repeat excision procedure should be carried out to confirm that the CIN has been excised completely and to confirm also that there are no further satellite foci of invasive disease. This should be carried out even in those cases planned for simple hysterectomy, in order to exclude an occult invasive lesion requiring radical surgery.

6.7 Complications after treatment of CIN

Complications after conservative therapy have been reported, but these are uncommon. In the short term there may be bleeding, discharge and infection. Long-term complications include cervical stenosis, and cervical insufficiency causing mid-trimester abortions. The latter complications are generally associated with knife conisation (Luesley *et al.*, 1985). Nevertheless, a recent systematic review indicated that all excisional procedures are associated with an increased frequency of low-

birth weight and premature delivery when compared to women who never had cervical treatment (Kyrgiou *et al.*, 2006). Stenosis and unsatisfactory colposcopy and cytological follow-up are complications usually due to the use of haemostatic sutures (Martin-Hirsch *et al.*, 2000). Rarely the cervix will be stenosed completely in which case in premenopausal women haematometra will occur, and the efficacy of follow-up cytology may be compromised: in post-menopausal women, there is a further problem in that it will be impossible to rely on the presence of post-menopausal bleeding to suspect invasive endometrial carcinoma. Complete cervical stenosis is also a problem for women having hormone replacement therapy (HRT). They will need to use daily progestogen to suppress endometrial proliferation due to oestrogen.

6.8 Follow-up after treatment of CIN

In terms of success or failure, there is no obviously superior conservative surgical technique for the treatment of CIN (Martin-Hirsch *et al.*, 2000). All women treated for CIN, whether CIN 1, 2 or 3, require regular follow-up. Excisional treatment procedures have the obvious advantage that they permit histological assessment of the biopsy. Histological examination of the entire TZ allows evaluation of the marginal status and exclusion of microinvasive or glandular disease. Women at increased risk of residual or recurrent disease should be considered for more intensive surveillance following treatment. Therefore, responsibility of the completeness of follow-up, using the intervals indicated below, needs to be clearly defined within the management process.

Some factors may influence the frequency and duration of follow-up:

1. Patient's age: women aged 40 or over are at increased risk of persistent or recurrent disease.
2. Type of lesion: glandular disease requires careful post operative assessment of the endocervical canal, usually with an endo-cervical brush sample.
3. Grade of lesion: high-grade lesions are more likely to persist or recur.
4. Histology of excised margins (suspicion of incomplete excision).

Women treated for **high-grade disease** (CIN2, CIN3, CGIN) require 6, 12 and 24-month follow-up cytology and thereafter annual cytology for a further 5 years before returning to screening at routine interval. Colposcopy is performed in addition to cytology at the 6-month follow-up visit (NHSCSP, 2004a; Nieminen *et al.*, 2006). Most persistent/recurrent disease is detected within the first 24 months (Chew *et al.*, 1999; Flannelly *et al.*, 2001). However, there is clear evidence that there is persistent long-term risk of invasive cancer for ten years after treatment (Soutter *et al.*, 1997).

Women treated for **low-grade disease** require 6-, 12-, 24-month follow-up cytology. If all results are negative, then women may be returned to screening at a routine interval.

Women treated for AIS are at higher risk of developing recurrent disease than those with high-grade CIN (Soutter *et al.*, 2001).

There is no clear evidence suggesting that the diagnostic performance of cytology in combination with colposcopy for the detection of persistent disease after treatment for CIN is superior to cytology alone. Some authors suggest that colposcopy does not increase the detection of disease (Gardeil *et al.*, 1997). Other authors (Mahadevan & Horwell, 1993; Flannelly *et al.*, 1997; Baldauf *et*

et al., 1998) suggest that an initial follow-up colposcopy marginally enhances early detection of disease and reduces the false negative rate.

6.8.1 Significance of involved margins in the excised specimen

Several retrospective studies (Andersen *et al.*, 1990; Murdoch *et al.*, 1992; Lopes *et al.*, 1993; Majeed *et al.*, 1994; Moore *et al.*, 1995; Chang *et al.*, 1996; Gold *et al.*, 1996; Gardeil *et al.*, 1997; Dobbs *et al.*, 2000; Flannelly *et al.*, 2001) of residual disease rates after LLETZ or knife cone biopsy have demonstrated that negative excision margins are associated with a lower risk of residual disease. Studies have demonstrated that disease at the endo-cervical resection margin is associated with increased risk of residual disease compared with involved ectocervical margins (Ostergard, 1980; Walton *et al.*, 1980; Schantz & Thormann, 1984; Boonstra *et al.*, 1990; Murdoch *et al.*, 1992; Lapaquette *et al.*, 1993; Lopes *et al.*, 1993; Gardeil *et al.*, 1997; Flannelly *et al.*, 2001). Women aged 40 or more (Paraskevaidis *et al.*, 2000; Flannelly *et al.*, 2001) are particularly at risk of persistent or recurrent disease.

All women over the age of 50 years who have CIN3 at the endo-cervical margin and in whom satisfactory cytology and colposcopy cannot be guaranteed should have a repeat excision to try to obtain clear margins.

If the pathologist has reported incomplete endo-cervical excision then an endo-cervical cytology sample is recommended.

6.8.2 The role of HPV testing in follow-up after treatment

The study of the sensitivity and specificity of HPV DNA testing to predict residual or recurrent neoplasia after treatment of CIN was the object of two recent systematic reviews (see Chapter 3) (Paraskevaidis *et al.*, 2004; Zielinski *et al.*, 2004). The first systematic review concluded that there is evidence that HPV testing post treatment can more quickly and efficiently detect a treatment failure than follow-up cytology. Zielinski reached similar conclusions. The data included in both studies were extended with newly published studies, and a formal meta-analysis was conducted (Arbyn *et al.*, 2005; Arbyn *et al.*, 2006). From this meta-analysis it was concluded that HPV DNA detection predicted residual/recurrent CIN with significantly higher sensitivity (ratio: 1.27; 95% CI: 1.06-1.51) and not-significantly lower specificity (ratio: 0.94; 95% CI: 0.87-1.01) than follow-up cytology. HPV DNA testing was also more sensitive than histology of the section margins (ratio: 1.30; 95% CI: 1.05-1.62). HPV testing was even more specific but this difference in specificity was statistically insignificant.

6.8.3 Treatment of residual and recurrent lesions

The presence of residual disease warrants excision of the transformation zone although in skilled hands, destruction may be considered provided that the conditions relating to preoperative assessment are met. However, post-treatment recurrence frequently occurs in the endo-cervical canal where it is not colposcopically detectable and therefore not suitable for ablative therapy (Lopes *et al.*, 1990; Murdoch *et al.*, 1992).

6.9 Management of women in other clinical situations

There are several circumstances in which management and treatment may differ from the general recommendations given above. The following particular situations are distinguished:

1. Pregnant women
2. Adolescent women
3. Post-menopausal women
4. Hysterectomised women
5. Immunocompromised women
6. Discrepancy between cytology, colposcopy and histology.

6.9.1 Management of women with cytological abnormality in pregnancy

Smears in pregnancy

Taking a smear should be postponed for pregnant women with negative screening histories unless the last smear was more than 5 years ago. If a woman has been called for routine screening and she is pregnant, the smear should usually be deferred. If a previous smear was abnormal and in the interim the woman becomes pregnant then the follow-up should not be delayed.

Colposcopy in pregnancy

A woman who meets the criteria for colposcopy still needs colposcopy if she is pregnant. The primary aim of colposcopy for pregnant women is to exclude invasive disease and to defer biopsy and treatment until the woman has delivered. Women who have low-grade cytology and in whom the colposcopy excludes high-grade disease, simply have a repeat colposcopy/cytology test 3-4 months after delivery. Women with high-grade disease and in whom colposcopy has excluded suspicion of invasive disease, should be reviewed at intervals of 3 months with a view to a final assessment 3-4 months following delivery. At that time a decision should be made on whether treatment is required.

The safety of delaying treatment of pregnant women has been shown in a number of cohort and retrospective uncontrolled studies (Coppola *et al.*, 1997). The incidence of invasive cervical cancer in pregnancy is low and pregnancy itself does not have an adverse effect on the prognosis (Coppola *et al.*, 1997). The risk of progression of CIN3 is low in pregnancy and the spontaneous regression rate is high. One study reported a spontaneous regression rate of 69% after pregnancy for histologically proven CIN3 (Yost *et al.*, 1999).

If colposcopy has been performed during pregnancy, post-partum assessment of women with an abnormal smear or biopsy-proven CIN is essential. Excision biopsy in pregnancy cannot be considered therapeutic and these women should be seen for colposcopy post-partum.

Colposcopic evaluation of the pregnant woman requires a high degree of skill.

If invasive disease is suspected clinically or colposcopically, a biopsy adequate to make the diagnosis is essential. Cone, wedge and diathermy loop biopsies are all associated with a risk of haemorrhage (Robinson *et al.*, 1997) and such biopsies should be taken only where appropriate facilities to deal with haemorrhage are available. Punch biopsy suggesting only CIN cannot reliably exclude invasion.

6.9.2 Adolescent women

Invasive cervical carcinoma is virtually non-existent in adolescent women (Sasieni & Adams, 1999). The prevalence of transient HPV infection after coitarche is high (Collins *et al.*, 2002). Cervical screening in this age group may detect prevalent low-grade disease which might have resolved spontaneously if screening were started at a later age (Collins *et al.*, 2002). This could result in unnecessary attendances at colposcopy, with the resultant possible negative consequences of increased anxiety and possible over-treatment. In addition screening has not been shown to be effective at reducing the incidence of invasive cancer in women under twenty (Wright & Riopelle, 1984; Moscicki *et al.*, 2004; Boardman *et al.*, 2005; Sawaya, 2005).

6.9.3 Post menopausal women

The incidence of abnormal cytology is extremely low in women of this age group who have previously had negative cytology. An episode of post-menopausal bleeding warrants a complete gynaecological assessment, with a cytology test, but is not an indication for colposcopy.

6.9.4 Hysterectomised women

Women who have had a hysterectomy with CIN present are potentially at risk of developing vaginal intra-epithelial neoplasia and cancer. The incidence of vaginal intra-epithelial neoplasia (VAIN) following hysterectomy diagnosed with CIN is in the order of 1% in a series of 341 women (Gemmell *et al.*, 1990) with no subsequent cases of invasive disease. In a similar series of 177 women (Burghardt & Holzer, 1980) 4% developed VAIN, with 0.6% developing subsequent invasive disease. A meta-analysis of long-term results suggests that while recurrent intraepithelial disease is less common after hysterectomy for CIN than after local treatment of the cervix (522 vs. 1587 per 100,000 woman-years), the risk of invasive recurrence is similar in both groups (57 vs. 67 per 100,000 woman-years) (Soutter *et al.*, 2005).

There is no clear evidence that colposcopy increases the detection of disease on follow-up.

A possible guideline for post hysterectomy follow-up is as follows:

1. For women who have been on routine screening for at least 10 years but who have no CIN in the specimen, no vault cytology is required.
2. For women who have been on routine screening for less than 10 years, and who have no CIN in the cervix, a smear 6 and 18 months from the vault and no further cytology follow-up if both are negative.

3. For women who have had a hysterectomy for CIN for some particular reason, and in whom the CIN has been excised completely, there should be a smear 6 and 18 months after the hysterectomy. If follow-up cytology at 18 months is negative, no further cytology is necessary.
4. For women with incomplete or uncertain excision of CIN, follow-up should be conducted as if the cervix were still *in situ* (i.e. as for low and high-risk CIN).

6.9.5 Immuno-suppressed patients

Patients with immunodeficiency due to immune-suppressing medication, transplantation and all other forms of immunosuppression will have an increased frequency of CIN. The risk of progression to invasive disease is higher and the success rate of treatment is lower. Continued patient surveillance is needed. The prevalence of abnormal cervical cytology in the renal transplant population of around 15% represents a five-fold increase from the normal population (ter Haar-van Eck *et al.*, 1995). There is also an increased incidence of CIN in women with systemic lupus erythematosus treated with long-term chemotherapy (Dhar *et al.*, 2001).

There is debate as to whether immunosuppressed patients should be screened more frequently, and in some centres annual cytology combined with colposcopy is recommended.

6.9.6 HIV-positive women

Whereas the estimated prevalence of cervical disease in HIV seronegative women is approximately 3% (Schiffman & Brinton, 1995), a number of reports including cross sectional, case-control and cohort studies have indicated a greatly increased prevalence of squamous intraepithelial lesions, ranging between 20 and 40% (Mandelblatt *et al.*, 1992) in HIV-infected women.

Annual cytology should be performed with an initial colposcopy if resources permit. High-grade histologically-proven disease should be treated as the guidelines recommend for non-HIV patients.

6.9.7 Procedure in case of cyto-colposcopic discrepancies

Occasionally, following a high-grade abnormal Pap smear, the colposcopy is normal. Such women are at risk of having or developing subsequent CIN2+. In this situation, before assuming that either the Pap test is falsely positive or before systematically recommending a diagnostic cone biopsy or loop excision of the TZ, smears should be repeated, and the original cytology should be reviewed.

Should cytological abnormalities persist, a second colposcopy is required. The colposcopic examination must be performed under optimal conditions, if necessary after treatment of any inflammatory or infective condition of the lower genital tract or after oestrogenic preparation in post-menopausal women. Special attention must be given to identifying the SCJ junction. If the SCJ is visible and no colposcopic abnormality is apparent, the investigation should be completed by a detailed examination of the vagina. If again there is no obvious lesion, the endo-cervical canal should

be assessed as thoroughly as possible. If no abnormality can be seen, then the TZ should be excised in its entirety; this should be combined with an endo-cervical sampling.

If the SCJ is not visible, and no abnormality can be identified on the cervix or the vagina, then the TZ should be excised in its visible entirety and the lower third of the endo-cervical canal should also be removed. This should be followed by an ECC.

The management depends also on the severity of the cytological abnormality. With minor cytological abnormalities the risk of failing to detect a severe histological lesion is low provided colposcopic assessment, together with, if indicated, colposcopically directed biopsies and perhaps endo-cervical curettage, are all negative. However, when cytology is suggestive of high-grade disease the major problem is to eliminate high-grade CIN or an early invasive disease.

Ideally, all cases with discrepant high-grade cytology, colposcopy or histology findings should be discussed in a multi-disciplinary forum to optimise management.

6.10 Quality assurance of patient management

To achieve optimum results from cervical screening, quality assurance at all levels is important. Each national cervical screening programme should produce guidelines that are relevant to its own country or region.

The aim of quality assurance is to optimize compliance and effectiveness of patient management according to defined standards, to inform women, and to provide feedback to healthcare professionals and decision makers.

Multidisciplinary meetings involving the cytologist, the pathologist and the clinician should be encouraged in both public and private hospitals. These meetings are useful for discussing general cytology, pathology and colposcopy practice but are also useful for discussing unusual cases and where there is a discrepancy between results. Auditing of practice should be encouraged.

6.11 Measures to improve follow-up

There should be national or EU-agreed guidelines regarding management and follow-up. Fail-safe measures should be installed to maximise compliance of screen-positive women with follow-up recommendations (NHSCSP, 2004b). Formally agreed-upon instructions should be developed to monitor the outcome of screen-detected lesions (see below and Chapter 7). The purpose is to measure the accuracy of cytology and colposcopy, using histology as reference, and to evaluate follow-up compliance and treatment effectiveness.

6.11.1 Fail-safe measures to assure compliance with follow advice

The primary responsibility for ensuring completed care for a woman with an abnormal smear rests with the smear taker. However, support from other services involved in the cervical screening program is essential to maximise follow-up compliance. The following fail-safe measures should be in place:

1. An abnormal smear report should be clearly marked with the phrase "further action required".
A copy of the smear report must be sent to the smear taker and the patient's general practitioner if he or she is not the smear taker. The woman should receive a letter informing her of the smear result or advising her to contact her doctor within a specified time.
2. A check-list of all smears must be kept by the smear taker who must ensure that all results are collated and acted upon.
3. The cytology laboratory should check whether action has been taken on any abnormal smear reports that have been issued. The cytology laboratories should send out a reminder to the smear taker and/or general practitioner if no action has been taken within 6 months of issuing an abnormal smear report. Fail-safe procedures could be a task of the screening programme manager, who has access to screening registries.
4. Despite all attempts to ensure action is taken, some women will escape follow-up either because they refuse further investigation or because they cannot be traced. The names of such women should be given to the programme manager who should keep a record of the attempts that have been made to contact the women concerned.

6.11.2 Correlation of cytology findings with the final histological diagnosis

Efforts should be made to correlate the reported cytological abnormality with the histological outcome. Since the laboratory is the only common factor in the diagnosis and follow-up of women with abnormal cytology, it should be the responsibility of the cytology laboratory to collate this information. It could also be the responsibility of the programme manager, working in conjunction with the laboratories.

Where the original cellular changes have been minor, information of cytological regression will suffice. However, in those cases which require histological assessment and treatment, the original cytology should be correlated with the final histology (Suba *et al.*, 2004). This needs to be organised in a way such that the wish for quality improvement does not increase the risk of harm by over-diagnosis and over-treatment of the women. This correlation between cytology and histology is an important component of maintaining and improving the quality of the cytology screening programme (IARC, 2005).

6.12 Patient information

Each woman must be informed (verbally or written) about the screening test result.

Anxiety can be produced by the mere process of cervical screening (Marteau *et al.*, 1990) when an abnormality is found which requires referral for colposcopy or treatment (Gath *et al.*, 1995; Freeman-Wang *et al.*, 2001).

To allay anxiety, the following points should be considered:

1. Each woman should receive verbal and/or written information before and after a cervical smear is taken. She should be reassured that she will be informed of the result either verbally (if necessary by telephone) or in a written form.
2. Each woman should receive verbal and written information before colposcopy.
3. Counselling should be available as an integral part of colposcopy.
4. Women should receive an appropriately worded invitation for colposcopy with a contact name, telephone number and clinic times.
5. Information following the colposcopy visit should be given to the patient verbally by the person performing the colposcopy. She should be told that the results of any investigations will be communicated to her within a few weeks.
6. If the visit to the colposcopy clinic has involved treatment then the results of histology of the excisional biopsy or punch biopsy should be communicated to the patient within a few weeks.
7. Information should be made available to ethnic minority and refugee groups.

6.13 Data collection on treatment and follow up of screen-detected lesions

A recommended minimum set of indicators should be permanently monitored. The minimum set of indicators can be monitored by hand-collecting items described in Table 3 and 4, but the use of an audit system is highly recommended for practical reasons and because it facilitates homogeneous data recording. The potential benefits of audit are unlikely to be accomplished unless physicians (gynaecologists) take responsibility for it and see it as an opportunity for permanent education and professional improvement rather than an attempt to control their activity.

Follow-up of the outcome (e.g. cancer or residual pre-cancerous lesion after treatment of a pre-cancerous lesions, and deaths, and survival rates after cancer treatments) also must be included in the auditing process. Systematic outcome data can be acquired by linking the treatment information, e.g. operation and diagnosis codes, with cancer registry or death records.

Table 3. Data to be collected on the treatment of lesions, and to be obtained from the cancer registry in case of occurrence of cancer

Personal identification
• personal identifier • date of birth
Diagnosis
• date of diagnosis • diagnosis and diagnosis code • stage • grade
Treatment
• date of treatment • treating physician • hospital code • operation code - radiotherapy - chemotherapy - radical hysterectomy - total hysterectomy - amputation of cervix - conisation/excision of the TZ - LLETZ - NETZ - laser - cold knife - local destructive therapy - laser vaporisation - cryotherapy - radical diathermy - cold coagulation
Compliance with
• treatment follow-up

Table 4. Carcinoma cases occurring during follow-up after treatment (from cancer registry and mortality records)

- personal identifier
- date of diagnosis of cancer
- diagnosis code - stage - grade
- vital status of the patient
- cause of death

6.14 References

- Abdel-Hady E.S., Martin-Hirsch P., Duggan-Keen M., Stern P.L., Moore J.V., Corbitt G., Kitchener H.C., & Hampson I.N. (2001). Immunological and viral factors associated with the response of vulval intraepithelial neoplasia to photodynamic therapy. *Cancer Res.* **61**: 192-196.
- Andersen E.S., Nielsen K., & Larsen G. (1990). Laser conization: follow-up in patients with cervical intraepithelial neoplasia in the cone margin. *Gynecol. Oncol.* **39**: 328-31.
- Andersen W., Frierson H., Barber S., Tabbarah S., Taylor P., & Underwood P. (1988). Sensitivity and specificity of endocervical curettage and the endocervical brush for the evaluation of the endocervical canal. *Am. J. Obstet. Gynecol.* **159**: 702-707.
- Anderson D.J., Strachan F., & Parkin D.E. (1992). Cone biopsy: Has endocervical sampling a role? *Br J Obstet Gynaecol* **99**: 668-670.
- Anderson M.C. & Hartley R.B. (1980). Cervical crypt involvement by intraepithelial neoplasia. *Obstet. Gynecol.* **55**: 546-50.
- Anderson M.C., Jordan J.A., Morse A.R., & Sharpe F. (1996). Integrated Colposcopy, 2nd edn. Chapman and Hall.
- Arbyn M., Albertyn G., & De Groof V. (1996). Consensus on follow-up recommendations in cervical cancer screening (in Dutch). *Tijdschr voor Geneeskunde* **52**: 709-715.
- Arbyn M., Buntinx F., Van Ranst M., Paraskevaidis E., Martin-Hirsch P., & Dillner J. (2004a). Virologic versus cytologic triage of women with equivocal Pap smears: a meta-analysis of the accuracy to detect high-grade intraepithelial neoplasia. *J. Natl. Cancer Inst.* **96**: 280-293.
- Arbyn M., Dillner J., Van Ranst M., Buntinx F., Martin-Hirsch P., & Paraskevaidis E. (2004b). Re: Have we resolved how to triage equivocal cervical cytology? *J.Natl.Cancer Inst.* **96**: 1401-1402.
- Arbyn M., Paraskevaidis E., Martin-Hirsch P., Prendiville W., & Dillner J. (2005). Clinical utility of HPV DNA detection: triage of minor cervical lesions, follow-up of women treated for high-grade CIN. An update of pooled evidence. *Gynecol. Oncol.* **99 S3**: 7-11.
- Arbyn M., Sasieni P., Meijer C.J., Clavel C., Koliopoulos G., & Dillner J. (2006). Chapter 9: Clinical applications of HPV testing: A summary of meta-analyses. *Vaccine* **24 S3**: 78-89.
- ASCUS-LSIL Triage Study Group (2003a). A randomized trial on the management of low-grade squamous intraepithelial lesion cytology interpretations. *Am. J. Obstet. Gynecol.* **188**: 1393-1400.
- ASCUS-LSIL Triage Study Group (2003b). Results of a randomized trial on the management of cytology interpretations of atypical squamous cells of undetermined significance. *Am. J. Obstet. Gynecol.* **188**: 1383-1392.
- Baldauf J.J., Dreyfus M., Ritter J., Cuenin C., Tissier I., & Meyer P. (1998). Cytology and colposcopy after loop electrosurgical excision: implications for follow-up. *Obstet. Gynecol.* **92**: 124-30.
- Bergeron C., Jeannel D., Poveda J., Cassonnet P., & Orth G. (2000). Human papillomavirus testing in women with mild cytologic atypia. *Obstet. Gynecol.* **95**: 821-827.

Boardman L.A., Stanko C., Weitzen S., & Sung J. (2005). Atypical squamous cells of undetermined significance: human papillomavirus testing in adolescents. *Obstet. Gynecol.* **105**: 741-746.

Bolger B.S. & Lewis B.V. (1988). A prospective study of colposcopy in women with mild dyskariosis or koilocytosis. *Br J Obstet Gynaecol* **95**: 1117-1119.

Boonstra H., Aalders J.G., Koudstaal J., Oosterhuis J.W., & Janssens J. (1990). Minimum extension and appropriate topographic position of tissue destruction for treatment of cervical intraepithelial neoplasia. *Obstet. Gynecol.* **75**: 227-231.

Burghardt E. & Holzer E. (1980). Treatment of carcinoma in situ: evaluation of 1609 cases. *Obstet. Gynecol.* **55**: 539-45.

Burke L., Covell L., & Antonioli D. (1980). Carbon dioxide laser therapy of cervical intraepithelial neoplasia: factors determining success rate. *Lasers Surg. Med.* **1**: 113-22.

Cartier R. & Cartier I. (1993). *Practical Colposcopy*, 3rd edn. Paris.

Chanen W. & Rome R.M. (1983). Electrocoagulation diathermy for cervical dysplasia and carcinoma in situ: a 15-year survey. *Obstet. Gynecol.* **61**: 673-679.

Chang D.Y., Cheng W.F., Torng P.L., Chen R.J., & Huang S.C. (1996). Prediction of residual neoplasia based on histopathology and margin status of conization specimens. *Gynecol. Oncol.* **63**: 53-56.

Chew G.K., Jandial L., Paraskevaidis E., & Kitchener H.C. (1999). Pattern of CIN recurrence following laser ablation treatment: long-term follow-up. *Int. J. Gynecol. Cancer* **9**: 487-490.

Coleman D., Day N., Douglas G., Farmery E., Lynge E., Philip J., & Segnan N. (1993). European Guidelines for Quality Assurance in Cervical Cancer Screening. Europe against cancer programme. *Eur J Cancer* **29A Suppl 4**: S1-S38.

Collins S., Mazloomzadeh S., Winter H., Blomfield P., Bailey A., Young L.S., & Woodman C.B. (2002). High incidence of cervical human papillomavirus infection in women during their first sexual relationship. *BJOG* **109**: 96-98.

Coppola A., Sorosky J., Casper R., Anderson B., & Buller R.E. (1997). The clinical course of cervical carcinoma in situ diagnosed during pregnancy. *Gynecol. Oncol.* **67**: 162-165.

Cox J.T., Schiffman M.A., & Solomon D. (2003). Prospective follow-up suggests similar risk of subsequent cervical intraepithelial neoplasia grade 2 or 3 among women with cervical intraepithelial neoplasia grade 1 or negative colposcopy and directed biopsy. *Am. J. Obstet. Gynecol.* **188**: 1406-1412.

Cuzick J., Szarewski A., Cubie H., Hulman G., Kitchener H., Luesley D., McGoogan E., Menon U., Terry G., Edwards R., Brooks C., Desai M., Gie C., Ho L., Jacobs I., Pickles C., & Sasieni P. (2003). Management of women who test positive for high-risk types of human papillomavirus: the HART study. *Lancet* **362**: 1871-1876.

Denny L., Kuhn L., De Souza M., Pollack A.E., Dupree W., & Wright T.C., Jr. (2005). Screen-and-treat approaches for cervical cancer prevention in low-resource settings: a randomized controlled trial. *JAMA* **294**: 2173-2181.

Dhar J.P., Kmak D., Bhan R., Pishorodi L., Ager J., & Sokol R.J. (2001). Abnormal cervicovaginal cytology in women with lupus: a retrospective cohort study. *Gynecol. Oncol.* **82**: 4-6.

Dobbs S.P., Asmussen T., Nunns D., Hollingworth D., Brown L.J., & Ireland D. (2000). Does histological incomplete excision of cervical intraepithelial neoplasia following large loop excision of transformation zone increase recurrence rates? A six year cytological follow-up. *BJOG* **107**: 1298-1301.

Duska L.R., Flynn C.F., Chen A., Whall-Strojwas D., & Goodman A. (1998). Clinical evaluation of atypical glandular cells of undetermined significance on cervical cytology. *Obstet. Gynecol.* **91**: 278-282.

Eddy G.L., Strumpf K.B., Wojtowycz M.A., Piraino P.S., & Mazur M.T. (1997). Biopsy findings in five hundred thirty-one patients with atypical glandular cells of uncertain significance as defined by the Bethesda system. *Am. J. Obstet. Gynecol.* **177**: 1188-1195.

Ferris D.G., Wright T.C., Litaker M.S., Richart R.M., Lorincz A.T., Sun X.W., & Woodworth C.D. (1998). Comparison of two tests for detecting carcinogenic HPV in women with Papanicolaou smear reports of ASCUS and LSIL. *J Fam Pract* **46**: 136-141.

Flannelly G., Bolger B., Fawzi H., De Lopes A.B., & Monaghan J.M. (2001). Follow-up after LLETZ: could schedules be modified according to risk of recurrence? *BJOG* **108**: 1025-1030.

Flannelly G., Langan H., Jandial L., Mana E., Campbell M., & Kitchener H. (1997). A study of treatment failures following large loop excision of the transformation zone for the treatment of cervical intraepithelial neoplasia. *Br J Obstet Gynaecol* **104**: 718-722.

Freeman-Wang T., Walker P., Linehan J., Coffey C., Glasser B., & Sherr L. (2001). Anxiety levels in women attending colposcopy clinics for treatment for cervical intraepithelial neoplasia: a randomised trial of written and video information. *BJOG* **108**: 482-4.

Fu Y.S., Reagan J.W., & Richart R.M. (1981). Definition of precursors. *Gynecol. Oncol.* **12**: 220-231.

Gaffikin L., Blumenthal P.D., Emerson M., & Limpaphayom K. (2003). Safety, acceptability, and feasibility of a single-visit approach to cervical-cancer prevention in rural Thailand: a demonstration project. *Lancet* **361**: 814-820.

Galvin G.A., Jones H.W., & Te Linde R.W. (1955). The significance of basal-cell hyperactivity in cervical biopsies. *Am. J. Obstet. Gynecol.* **70**: 808-817.

Gardeil F., Barry-Walsh C., Prendiville W., Clinch J., & Turner M.J. (1997). Persistent intraepithelial neoplasia after excision for cervical intraepithelial neoplasia grade III. *Obstet. Gynecol.* **89**: 419-422.

Gath D.H., Hallam N., Mynors-Wallis L., Day A., & Bond S.A.K. (1995). Emotional reactions in women attending a UK colposcopic clinic. *J. Epidemiol. Community Health* **49**: 79-83.

Gemmell J., Holmes D.M., & Duncan I.D. (1990). How frequently need vaginal smears be taken after hysterectomy for cervical intraepithelial neoplasia? *Br J Obstet Gynaecol* **97**: 58-61.

Gold M., Dunton C.J., Murray J., Macones G., Hanau C., & Carlson J.A., Jr. (1996). Loop electrocautery excisional procedure: therapeutic effectiveness as an ablation and a conization equivalent. *Gynecol. Oncol.* **61**: 241-4.

Guido R., Schiffman M.A., Solomon D., & Burke L. (2003). Postcolposcopy management strategies for women referred with low-grade squamous intraepithelial lesions or human papillomavirus DNA-positive atypical squamous cells of undetermined significance: a two-year prospective study. *Am. J. Obstet. Gynecol.* **188**: 1401-1405.

Hinselmann H. (1925). Verbesserung der Inspektionsmöglichkeiten von Vulva, Vagina und Portio. *Münchener Med. Wochenschr.* **72**: 1733-1742.

Hoffman M.S., Sterghos S.Jr., Gordy L.W., & Gunasekar D. (1993). Evaluation of the cervical canal with the endocervical brush. *Obstet. Gynecol.* **82**: 573-577.

Holowaty P., Miller A.B., Rohan T., & To T. (1999). Natural History of Dysplasia of the Uterine Cervix. *J. Natl. Cancer Inst.* **91**: 252-258.

IARC (2005). Cervix Cancer Screening. IARC Handbooks of Cancer Prevention. Vol. 10. IARC Press, Lyon

Ismail S.M., Colclough A.B., Dinnen J.S., Eakins D., Evans D.M., Gradwell E., O'Sullivan J.P., Summerell J.M., & Newcombe R.G. (1989). Observer variation in histopathological diagnosis and grading of cervical intraepithelial neoplasia [see comments]. *BMJ* **298**: 707-710.

Jordan J.A. (1985). Colposcopy in the diagnosis of cervical cancer and precancer. *Clin. Obstet. Gynecol.* **12**: 67-76.

Kennedy A.W., Salmieri S.S., Wirth S.L., Biscotti C.V., Tuason L.J., & Travarca M.J. (1996). Results of the clinical evaluation of atypical glandular cells of undetermined significance (AGCUS) detected on cervical cytology screening. *Gynecol. Oncol.* **63**: 14-18.

Kim T.J., Kim H.S., Park C.T., Park I.S., Hong S.R., Park J.S., & Shim J.U. (1999). Clinical evaluation of follow-up methods and results of atypical glandular cells of undetermined significance (AGUS) detected on cervicovaginal Pap smears. *Gynecol. Oncol.* **73**: 292-298.

Kurman R.J., Henson D.E., Herbst A.L., Noller K.L., Schiffman M.H., & National Cancer Institute (1994). Interim guidelines for management of abnormal cervical cytology. *JAMA* **271**: 1866-1869.

Kyrgiou M., Koliopoulos G., Martin-Hirsch P., Arbyn M., Prendiville W., & Paraskevidis E. (2006). Obstetric outcomes after conservative treatment for intra-epithelial or early invasive cervical lesions: a systematic review and meta-analysis of the literature. *Lancet* **367**: 489-498.

Koliopoulos G., Arbyn M., Martin-Hirsch P., Kyrgiou M., Prendiville W., & Paraskevidis E. (2007). Diagnostic accuracy of human papillomavirus testing in primary cervical screening: a systematic review and meta-analysis of non randomised studies. *Gynecol. Oncol.* **104**: 232-246

Lambert B. & Woodruff D.J. (1963). Spinal cell atypia of the cervix. *Cancer* **16**: 1141-1150.

Lange P. (1960). Clinical and histological studies on cervical carcinoma. Precancerosis, early metastases and tubular structures in the lymph nodes. *Acta Pathol. Microbiol. Scand.* **50 (Suppl 143)**: 1-179.

Lapaquette T.K., Dinh T.V., Hannigan E.V., Doherty M.G., Yandell R.B., & Buchanan V.S. (1993). Management of patients with positive margins after cervical conization. *Obstet. Gynecol.* **82**: 440-3.

MANAGEMENT OF ABNORMAL CERVICAL CYTOLOGY

- Lopes A., Mor-Yosef S., Pearson S., Ireland D., & Monaghan J.M. (1990). Is routine colposcopic assessment necessary following laser ablation of cervical intraepithelial neoplasia? *Br J Obstet Gynaecol* **97**: 175-7.
- Lopes A., Morgan P., Murdoch J., Piura B., & Monaghan J.M. (1993). The case for conservative management of "incomplete excision" of CIN after laser conization. *Gynecol. Oncol.* **49**: 247-9.
- Luesley D.M., McCrum A., Terry P.B., Wade Evans T., Nicholson H.O., Mylotte M.J., Emens J.M., & Jordan J.A. (1985). Complications of cone biopsy related to the dimensions of the cone and the influence of prior colposcopic assessment. *Br J Obstet Gynaecol* **92**: 158-164.
- Mahadevan N. & Horwell D.H. (1993). Histological incomplete excision of cin after large loop excision of the transformation zone (lletz) merits careful follow-up, not retreatment [letter]. *Br J Obstet Gynaecol* **100**: 794-795.
- Majeed F.A., Cook D.G., Anderson H.R., Hilton S., Bunn S., & Stones C. (1994). Using patient and general practice characteristics to explain variations in cervical smear uptake rates. *BMJ* **308**: 1272-1276.
- Mandelblatt J.S., Fahs M., Garibaldi K., Senie R.T., & Peterson H.B. (1992). Association between HIV infection and cervical neoplasia: implications for clinical care of women at risk for both conditions. *AIDS* **6**: 173-178.
- Marteau T.M., Walker P., Giles J., & Smail M. (1990). Anxieties in women undergoing colposcopy. *Br J Obstet Gynaecol* **97**: 859-61.
- Martin M. & Prendiville W. (2004). Is local anaesthetic infiltration less painful than cervical punch biopsy. A study comparing the amount of pain felt by women attending a colposcopy clinic who required a cervical punch biopsy. British Society of Colposcopy and Cervical Pathology. Edinburgh. Annual Meeting.
- Martin-Hirsch P., Paraskevaidis E., & Kitchener H. (2000). Surgery for cervical intraepithelial neoplasia. *Cochrane Database Syst Rev*.
- Massad L.S., Halperin C.J., & Bitterman P. (1996). Correlation between colposcopically directed biopsy and cervical loop excision. *Gynecol. Oncol.* **60**: 400-403.
- Melnikow J., Nuovo J., Willan A.R., Chan B.K., & Howell L.P. (1998). Natural History of cervical squamous intraepithelial lesions : a meta-analysis. *Obstet. Gynecol.* **92**: 727-735.
- Mogensen S.T., Bak M., Dueholm M., Frost L., Knoblauch N.O., Praest J., & Svanholm H. (1997). Cytobrush and endocervical curettage in the diagnosis of dysplasia and malignancy of the uterine cervix. *Acta Obstet Gynecol Scand* **76**: 69-73.
- Moore B.C., Higgins R.V., Laurent S.L., Marroum M.C., & Bellitt P. (1995). Predictive factors from cold knife conization for residual cervical intraepithelial neoplasia in subsequent hysterectomy. *Am. J. Obstet. Gynecol.* **173**: 361-368.
- Mor-Yosef S., Lopes A., Pearson S., & Monaghan M. (1990). Loop diathermy cone biopsy. *Obstet. Gynecol.* **73**: 884-886.
- Morin C., Bariati C., Bouchard C., Fortier M., Roy M., Moore L., & Meisels A. (2001). Managing Atypical Squamous Cells of Undetermined Significance in Papanicolaou Smears. *J. Reprod. Med.* **46**: 799-805.

Moscicki A.B., Shiboski S., Hills N.K., Powell K.J., Jay N., Hanson E.N., Miller S., Canjura-Clayton K.L., Farhat S., Broering J.M., & Darragh T.M. (2004). Regression of low-grade squamous intra-epithelial lesions in young women. *Lancet* **364**: 1678-1683.

Murdoch J.B., Morgan P.R., Lopes A., & Monaghan J.M. (1992). Histological incomplete excision of CIN after large loop excision of the transformation zone (LLETZ) merits careful follow-up, not retreatment. *Br. J. Obstet. Gynaecol.* **99**: 990-993.

Nieminen P, Anttila A, Bützow R, Heikkilä E, Hiltunen-Back E, Puistola U, Rantanen V, Räisänen I, Santalahti A, Talvensaari-Mattila A, Vartiainen J, Vuento M, Yliskoski M. (2006). Guideline for the diagnosis and treatment of pre-cancerous lesions of the cervix uteri, vulva, and vagina. Duodecim & the Finnish Association of Colposcopists (www.kaypahoito.fi)

NHSCSP (1996a). Standards and quality in colposcopy. Luesley, D. 2, 1-27. Sheffield, NHS Cancer Screening Programmes. NHSCSP Publications.

NHSCSP (1996b). The Colposcopy Examination. Cancer Research UK.

NHSCSP (1997). Guidance notes on the safe use of diathermy loop excision for the treatment of cervical intraepithelial neoplasia. Hancock, C. W. NHSCSP publication 4, 1-10. Sheffield, NHS Cancer Screening Programmes. NHSCSP Publications.

NHSCSP (2004a). Colposcopy and programme management: guidelines for the NHS Cervical Screening Programme. Luesley, D. and Leeson, S. NHSCSP publication 20, 1-80. Sheffield, Manor House. NHS Cancer Screening Programmes.

NHSCSP (2004b). Guidelines on failsafe actions for the follow-up of cervical cytology reports. NHSCSP publication 21, -20. NHSCSP Publications.

Nobbenhuis M.A., Helmerhorst T.J., van den Brule A.J., Rozendaal L., Voorhorst F.J., Bezemer P.D., Verheijen R.H., & Meijer C.J. (2001). Cytological regression and clearance of high-risk human papillomavirus in women with an abnormal cervical smear. *Lancet* **358**: 1782-1783.

Nobbenhuis M.A.E., Walboomers J.M., Helmerhorst T.J.M., Rozendaal L., Remmink A.J., Risse E.K.J., van der Linden H.C., Voorhorst F.J., Kenemans P., & Meijer C.J.L.M. (1999). Relation of human papillomavirus status to cervical lesions and consequences for cervical-cancer screening : a prospective study. *Lancet* **354**: 20-25.

Nygard J.F., Skare G.B., & Thoresen S.O. (2002). The cervical cancer screening programme in Norway, 1992-2000: changes in Pap smear coverage and incidence of cervical cancer. *J. Med. Screen.* **9**: 86-91.

Ostergard D.R. (1980). Cryosurgical treatment of cervical intraepithelial neoplasia. *Obstet. Gynecol.* **56**: 231-233.

Pahl I.R., Stein A.A., Rome D., & Plotz E.J. (1965). Basal cell proliferative disease of the cervix : a diagnostic approach. *Obstet. Gynecol.* **25**: 201-208.

Paraskevaidis E., Arbyn M., Diakomanolis E., Martin-Hirsch P., Koliopoulos G., Makrydimas G., Tofoski J., & Roukos D. (2004). The role of HPV DNA testing in the follow-up period after treatment for CIN: a systematic review of the literature. *Cancer Treat Rev* **30**: 205-211.

Paraskevaïdis E., Lolis E.D., Koliopoulos G., Alamanos Y., Fotiou S., & Kitchener H.C. (2000). Cervical intraepithelial neoplasia outcomes after large loop excision with clear margins. *Obstet. Gynecol.* **95**: 828-831.

Peckham B. & Greene R.R. (1957). Follow-up on cervical epithelial abnormalities. *Am. J. Obstet. Gynecol.* **74**: 804-815.

Prendiville W. (2003a). LLETZ: theoretical rationale, practical aspects, clinical experience, optimizing the technique. In: *Colposcopy: Management Options* (eds Prendiville W., Ritter J., Tatti S. & Twiggs L.), pp. 75-89. Saunders, Edinburgh.

Prendiville W. (2003b). The treatment of grade 3 cervical intraepithelial neoplasia. In: *Colposcopy: management options* (eds Prendiville W., Ritter J., Tatti S. & Twiggs L.B.), pp. 129-133. Saunders, Edinburgh.

Prendiville W., Davies R., & Berry P.J. (1986). A low voltage diathermy loop for taking cervical biopsies. A qualitative comparison with punch biopsy forceps. *Br. J. Obstet. Gynaecol.* **93**: 773-776.

Prendiville W., Ritter J., Tatti S., & Twiggs L. (2003). *Colposcopy: management options*. Saunders.

Pretorius R.G., Belinson J.L., Zhang W.H., Burchette R.J., Elson P., & Qiao Y.L. (2001). The colposcopic impression. Is it influenced by the colposcopist's knowledge of the findings on the referral Papanicolaou smear? *J. Reprod. Med.* **46**: 724-728.

Pretorius R.G., Zhang W.H., Belinson J.L., Huang M.N., Wu L.Y., Zhang X., & Qiao Y.L. (2004). Colposcopically directed biopsy, random cervical biopsy, and endocervical curettage in the diagnosis of cervical intraepithelial neoplasia II or worse. *Am. J. Obstet. Gynecol.* **191**: 430-434.

Remmink A.J., Walboomers J.M., Helmerhorst T.J.M., Voorhorst F.J., Rozendaal L., Risse E.K.J., Meijer C.J.L.M., & Kenemans P. (1995). The presence of persistent high-risk HPV genotypes in dysplastic cervical lesions is associated with progressive disease: natural history up to 36 months. *Int. J. Cancer* **61**: 306-311.

Robinson W.R., Webb S., Tirpack J., Degefu S., & O'Quinn A.G. (1997). Management of cervical intraepithelial neoplasia during pregnancy with LOOP excision. *Gynecol. Oncol.* **64**: 153-155.

Ronnett B.M., Manos M., Ransley J.E., Fetterman B., Kinney W., Hurley A., Ngai J.S., Kurman R., & Sherman M.E. (1999). Atypical glandular cells of undetermined significance (AGUS) : cytopathologic features, histopathologic results, and human papillomavirus DNA detection. *Hum Pathol* **30**: 816-825.

Sasieni P. & Adams J. (1999). Effect of screening on cervical cancer mortality in England and Wales: analysis of trends with an age period cohort model. *BMJ* **318**: 1244-1245.

Sawaya G.F. (2005). A 21-year-old woman with atypical squamous cells of undetermined significance. *JAMA* **294**: 2210-2218.

Schantz A. & Thormann L. (1984). Cryosurgery for dysplasia of the uterine ectocervix. A randomized study of the efficacy of the single- and double-freeze techniques. *Acta Obstet Gynecol Scand* **63**: 417-420.

Schiffman M.H. & Brinton L.A. (1995). The epidemiology of cervical carcinogenesis. *Cancer* **76**: 1888-1901.

Schlecht N.F., Platt R.W., Duarte-Franco E., Costa M.C., Sobrinho J.P., Prado J.C., Ferenczy A., Rohan T.E., Villa L.L., & Franco E.L. (2003). Human papillomavirus infection and time to progression and regression of cervical intraepithelial neoplasia. *J. Natl. Cancer Inst.* **95**: 1336-1343.

Sellers JW and Sankaranarayanan R. (2003). Colposcopy and Treatment of Cervical Intraepithelial Neoplasia: A Beginners' Manual. IARC Non serial publication, Lyon.

Solomon D., Davey D., Kurman R., Moriarty A., O'Connor D., Prey M., Raab S., Sherman M.E., Wilbur D., Wright T.C., & Young N. (2002). The 2001 Bethesda System: terminology for reporting results of cervical cytology. *JAMA* **287**: 2114-2119.

Solomon D., Schiffman M.A., & Tarone B. (2001). Comparison of three management strategies for patients with atypical squamous cells of undetermined significance (ASCUS): baseline results from a randomized trial. *J. Natl. Cancer Inst.* **93**: 293-299.

Soofer S.B. & Sidawy M.K. (2000). Atypical glandular cells of undetermined significance: clinically significant lesions and means of patient follow-up. *Cancer* **90**: 207-214.

Soutter W.P., de Barros Lopes A., Fletcher A., Monaghan J.M., Duncan I.D., Paraskevaides E., & Kitchener H.C. (1997). Invasive cervical cancer after conservative therapy for cervical intraepithelial neoplasia [see comments]. *Lancet* **349**: 978-980.

Soutter W.P., Haidopoulos D., Gornall R.J., McIndoe G.A., Fox J., Mason W.P., Flanagan A., Nicholas N., Barker F., Abrahams J., Lampert I., & Sarhanis P. (2001). Is conservative treatment for adenocarcinoma in situ of the cervix safe? *BJOG* **108**: 1184-1189.

Soutter W.P., Sasieni P., & Panoskaltsis T. (2005). Long-term risk of invasive cervical cancer after treatment of squamous cervical intraepithelial neoplasia. *Int. J. Cancer* **118**: 2048-2055.

Soutter W.P., Wisdom S., Brough A.K., & Monaghan J.M. (1986). Should patients with mild atypia in a cervical smear be referred for colposcopy? *Br J Obstet Gynaecol* **93**: 70-74.

Spitzer M., Chernys A.E., Shifrin A., & Ryskin M. (1998). Indications for cone biopsy: pathologic correlation. *Am. J. Obstet. Gynecol.* **178**: 74-9.

Stoler M.H. & Schiffman M.A. (2001). Interobserver Reproducibility of Cervical Cytologic and Histologic Interpretations. *JAMA* **285**: 1500-1505.

Suba E.J., Donnelly A.D., & Raab S.S. (2004). Crossing the quality chasm: a requirement for successful cervical cancer prevention in developing countries. Review. *Clin.Lab.Med.*

Taylor R.R., Guerrieri J.P., Nash J.D., Henry M.R., & O'Connor D.M. (1993). Atypical cervical cytology. Colposcopic follow-up using the Bethesda System. *J. Reprod. Med.* **38**: 443-447.

ter Haar-van Eck S.A., Rischen-Vos J., Chadha-Ajwani S., & Huikeshoven F.J. (1995). The incidence of cervical intraepithelial neoplasia among women with renal transplant in relation to cyclosporine. *Br J Obstet Gynaecol* **102**: 58-61.

Valdini A., Vaccaro C., Pechinsky G., & Abernathy V. (2001). Incidence and evaluation of an AGUS Papanicolaou smear in primary care. *J. Am. Board Fam. Pract.* **14**: 172-177.

Van Duin M., Snijders P.J., Schrijnemakers H.F., Voorhorst F.J., Rozendaal L., Nobbenhuis M.A., van den Brule A.J., Verheijen R.H., Helmerhorst T.J., & Meijer C.J. (2002). Human papillomavirus 16

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load in normal and abnormal cervical scrapes: an indicator of CIN II/III and viral clearance. *Int. J. Cancer* **98**: 590-595.

Walker P., Dexeus S., De Palo G., Barrasso R., Campion M., Girardi F., Jakob C., & Roy M. (2003). International terminology of colposcopy: an updated report from the International Federation for Cervical Pathology and Colposcopy. *Obstet. Gynecol.* **101**: 175-177.

Walton L.A., Edelman D.A., Fowler W.C., Jr., & Photopulos G.J. (1980). Cryosurgery for the treatment of cervical intraepithelial neoplasia during the reproductive years. *Obstet. Gynecol.* **55**: 353-7.

Weitzman G.A., Korhonen M.O., Reeves K.O., Irwin J.F., Carter T.S., & Kaufman R.H. (1988). Endocervical brush cytology. An alternative to endocervical curettage? *J. Reprod. Med.* **33**: 677-683.

Wright T.C., Cox J.T., Massad L.S., Carlson J., Twiggs L.B., & Wilkinson E.J. (2003). 2001 consensus guidelines for the management of women with cervical intraepithelial neoplasia. *Am. J. Obstet. Gynecol.* **189**: 295-304.

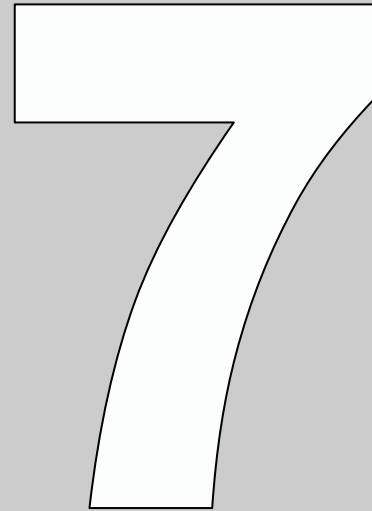
Wright T.C., Cox J.T., Massad L.S., & Wilkinson E.J. (2002). 2001 Consensus guidelines for the management of women with cervical cytological abnormalities. *JAMA* **287**: 2120-2129.

Wright T.C., Sun X.W., & Koulos J. (1995). Comparison of management algorithms for the evaluation of women with low-grade cytologic abnormalities. *Obstet. Gynecol.* **85**: 202-210.

Wright V.C. & Riopelle M.A. (1984). Age at beginning of coitus versus chronologic age as a basis for Papanicolaou smear screening: an analysis of 747 cases of preinvasive disease. *Am. J. Obstet. Gynecol.* **149**: 824-830.

Yost N.P., Santoso J.T., McIntire D.D., & Iliya F.A. (1999). Postpartum regression rates of antepartum cervical intraepithelial neoplasia II and III lesions. *Obstet. Gynecol.* **93**: 359-362.

Zielinski G.D., Bais A.G., Helmerhorst T.J., Verheijen R.H., De Schipper F.A., Snijders P.J., Voorhorst F.J., van Kemenade F.J., Rozendaal L., & Meijer C.J. (2004). HPV testing and monitoring of women after treatment of CIN 3: review of the literature and meta-analysis. *Obstet. Gynecol. Surv.* **59**: 543-553.



Key performance indicators

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7.1 Executive summary

A list of key performance indicators is provided for monitoring the screening process and for identifying and reacting to potential problems at an early time. The indicators address aspects of the screening process which influence the impact, as well as the human and financial costs of screening. Three groups of indicators can be distinguished:

1. **Screening intensity.** The proportion of the target population actually screened within the recommended interval is the main determinant of the success of a screening programme. However, too frequent testing increases financial and human costs with only marginal gain in reduction of incidence and mortality. The duration of the recommended screening interval must therefore be taken into account in monitoring and evaluating screening intensity. Indicators include: programme extension, compliance with invitation, coverage, and smear consumption.
2. **Screening test performance.** Essential indicators include the referral rates for repeat cytology and for colposcopy, as well as the positive predictive value of referral for colposcopy, the specificity of the screening test, and the rate of detection of histologically confirmed CIN.
3. **Diagnostic assessment and treatment.** Indicators include compliance to referral for repeat cytology and for colposcopy; treatment of high-grade lesions is also an essential performance indicator. The proportion of women hysterectomised for CIN serves as an indicator of extreme over-treatment.

Most of the key performance indicators can be directly computed from the tables presented in the annex of Chapter 2. However, a number of indicators are based on the incidence of invasive cervical cancers in women with different screening history. These indicators provide a more direct evaluation of the impact of screening, but they need to be computed over longer periods of time and linkage of screening registry data with cancer registry data is required for some indicators; see also section 5 in Chapter 2.

7.2 Screening intensity

Usually the most important factor contributing to the success of screening is **coverage**, i.e., the proportion of women in the target population actually screened at least once during the standard interval recommended by the screening programme (3 or 5 years). Measuring coverage directly requires computerised registration of all cytology and the capacity to link the findings of each woman individually. There can be problems regarding completeness of registration, in particular for tests performed outside an organised programme; in such cases estimates obtained from ad hoc surveys can be helpful. Coverage should be computed for the entire target age-group as defined by the national or regional screening policy, and also stratified by 5-year age group. Moreover, coverage should also be computed for the group of women aged 25-64, for whom evidence of screening effectiveness is most clear in almost all EU member states.

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In order to attain high screening coverage, it is necessary to reach the entire target population. This means that all women in the target population must be invited every three (or five) years, i.e. about one-third (or one-fifth) of the target population per year.

Compliance with invitation may be a less relevant parameter if opportunistic cervical screening is widespread. It should be kept in mind; however, that participation in organised screening programmes, as opposed to opportunistic screening, has resulted in the greatest decrease in the incidence of cervical cancer. Compliance provides a measure of the effectiveness of sending invitations, and, in addition, it provides a measure of the perceived quality of the programme.

A measure of test **consumption** is also essential. A large excess of smears per screened woman compared to the volume justified by the existing screening protocol has been observed in many countries. This is inefficient. As is the case for coverage, reliable measures of test consumption would require complete registration of smears. Underestimates can result from incompleteness of Registration, particularly for smears performed outside the organised screening programme. Estimates obtained from ad-hoc surveys can be helpful in such cases; health insurance agencies are an additional potential source of information.

The **incidence of invasive cervical cancer in unscreened and underscreened women, including women** never screened and women who were screened at intervals longer than that which is recommended by the local programme provides a direct measure of the burden of disease resulting from lack of coverage.

7.3 Screening test performance

The **rate of referral for repeat cytology** and the **referral rate for colposcopy** are measures not only of economic cost but also of the burden on women (anxiety, time consumption), which must be kept as low as possible. These rates depend on the sensitivity and the specificity of the screening test, and also on the prevalence of disease and on locally adopted protocols. The prevalence of disease is higher at the initial than at subsequent screening episodes. Therefore, these rates should be computed separately for women at initial, and subsequent screening episodes; and they also should be broken down by category of cytological abnormality that caused the referral.

The referral rate for repeat cytology due to unsatisfactory smears provides an approximation of the proportion of unsatisfactory smears resulting from poor quality smear taking.

The **positive predictive value (PPV) of referral for colposcopy** for detection of histologically confirmed high-grade CIN is calculated based on the actual number of women having colposcopies performed. This indicator readily shows the number of colposcopies which must be performed in order to find one lesion requiring treatment. (This number is the reciprocal of PPV).

Overall PPV for all women referred for colposcopy depends largely on the local protocol for colposcopy referral. This parameter should therefore be computed by cytological category and for different grades of CIN. PPV depends essentially on specificity (and to a minor extent on sensitivity) but is also strongly influenced by disease prevalence. Therefore it should also be computed separately for women attending initial and subsequent screening. Since PPV varies with the prevalence of disease, **test specificity** should also be computed; this will also permit comparison of performance of cytology interpretation between different screening programmes. Since specificity cannot

be calculated directly from screening programme data, the following formula can be used for approximation: number of women with negative screening test results / (number of screened women – number of women with confirmed CIN).

The **detection rate (DR) of CIN** (particularly of CIN2 and CIN3) depends on how many lesions are present in the screened population (i.e., on disease prevalence) and on how many of them are actually identified (cross sectional sensitivity). Since the prevalence of disease varies geographically and is a priori unknown, it is difficult to use the DR as an indicator of sensitivity. In addition, the DR also depends on the criteria of interpretation of histology, which are subject to variation. Nevertheless, DR should be monitored and compared between European screening programmes. This will provide a tool for recognising variation in quality and for developing the descriptive epidemiology of CIN in Europe which is needed for further study to improve control of cervical cancer.

Unfortunately, no easily interpretable indicator of screening sensitivity can be collected in a screening monitoring system. It is therefore essential to link screening registry and cancer registry data. Although it is difficult to obtain comparable data, in principle comparison of the **incidence of cancers** which are detected in women **after** having findings of **normal cytology** to the expected incidence in the absence of screening provides a direct estimate of test sensitivity for invasive lesions (see: Monitoring and evaluation, Chapter 2, Section 2.5). Information on cervical cancer incidence among unscreened women can be considered, if adjustments for selection bias in relation to screening attendance or non-attendance are made. Correspondingly, estimates of screening episode sensitivity may be obtained from inclusion of all screened women in the follow-up of cervical cancers. For programme sensitivity, it is essential to consider also women invited, but not screened. Previous smears of women with screen-detected cancer should also be reviewed (mixed with those of other women who did not develop cancer in order to avoid over-interpretation)

In addition to the above parameters, the distribution of the interval to reporting (time between smear taking and result communication) should be monitored. It seems implausible that reporting delays which are not extreme could influence screening effectiveness. Nevertheless, such delay influences women's perception of the quality of service, which affects participation and anxiety.

7.4 Diagnostic assessment and treatment

An important condition for the success of a screening programme is that diagnostic assessment is actually performed when needed. Measuring **compliance with referral for colposcopy** requires systematic and complete registration of colposcopies. When a record is lacking in the colposcopy register, the patient or her doctor should be contacted to obtain information on whether the colposcopy was performed and to remind about the need for examination. Compliance with colposcopy should be computed for each category of cytology that was the reason for referral (more severe cytology being of greatest relevance). Clearly, compliance will be higher for longer time spans after referral. Therefore, compliance should be monitored for different time intervals.

Another condition crucial to screening effectiveness is actual delivery of requisite treatment, particularly for histologically confirmed CIN2 and CIN3.

Avoiding over-treatment is the other important target. The proportion of women with pre-invasive lesions who undergo hysterectomy is a major indicator of unnecessary treatment, although some hysterectomies result from co-existing pathology. Peer-review should be conducted to verify the ap-

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appropriateness of treatment in such cases. It must be kept in mind that relevant differences in the proportion of women with CIN who undergo hysterectomy suggest that local practice is the main cause of such differences. Due to frequent spontaneous regression, only a small proportion of low-grade lesions should be treated.

Absence of SIL (or of high-risk HPV infection) can be routinely monitored at 6-month follow-up of treated women. This parameter has therefore been included as an indicator of short-term quality of treatment.

The **incidence of cervical cancer** in women which was not detected by screening, although the screening cytology results were abnormal (i.e., **after abnormal cytology**), serves as a direct summary indicator of failure associated with diagnostic assessment and treatment. Different reasons for failure can be distinguished. For example, cervical cancer arising in women who did not comply with referral for colposcopy represents a failure in communication. Cases arising in women who had colposcopy, but without detection of CIN, represent failure in diagnostic accuracy, etc. To calculate this parameter, the screening history of each case of cervical cancer should be reviewed (see also Chapter 2, section 5.3), and those cases should be excluded in which cancer was detected as a result of screening.

The present parameters assume that cytology is used as the primary screening test, which is currently recommended. However, most of the present parameters may also be applied, with only small changes, if a different screening method (e.g. HPV DNA testing) is used. Depending on the respective screening test and the screening policy, the values of some parameters (e.g., DR, PPV or specificity) may be expected to change.

7.5 Definition of performance parameters in cervical cancer screening

For general instructions on calculation of the following parameters, see sections 7.1 to 7.4. Specific instructions are indicated below and in the annex to Chapter 2, which is cross referenced in a number of the following descriptions of the performance parameters.

For short-term monitoring purposes, the calculations in the annex to Chapter 2 are based on annually aggregated data. Additional aggregation over different periods of time is recommended, particularly over the full screening interval of a given screening programme (3 or 5 years) and is required for some of the performance parameters. Wherever possible, longer and shorter evaluation periods should also be considered.

For calculations for a given period of time, such as the recommended screening interval (3 or 5 years), the dates on which the period starts and ends, and the procedure for determining the target population should be recorded. For calculations based on the size of the target population, use the average over the given time period.

Note that parameters 6 (Incidence of invasive cancer in unscreened women), 14 (Cancer incidence after normal cytology) and 19 (Incidence of invasive cancer after abnormal cytology) require **linkage with cancer registry data**. The follow-up periods recommended for calculation of cervical cancer incidence are six months longer than the recommended screening interval of the respective programme (3.5 or 5.5 years). The purpose of adding one-half year to the screening inter-

val is to include screen-detected cancer at the next screening episode. Calculations based on longer follow-up periods are also recommended.

7.5.1 Screening intensity

1. Programme extension

- Programme extension should be calculated regionally and nationally.
- If an entire region or country is actively served by a screening programme or programmes, then the programme extension in that region or country is 100%.

N women in target population
of catchment area
actively served by programme

N women in target population
of entire respective
region or country

2. Coverage of the target population by invitation

- Length of period corresponds to interval between two negative smear tests recommended by screening programme policy.
- Stratification by 5-year age groups is recommended.
- Obtain data from Table B1 in annex to Chapter 2. Also calculate separately using eligible women as denominator.
- For short-term monitoring, also calculate separately for women invited in the most recent calendar year in which screening was performed.
- For interpretation, take into account whether all women are invited or only a subset (**see Table A2** in annex to Chapter 2).

N women invited
in defined period (3 or 5 years)

N resident women in
target population

3. Coverage of the target population by smear tests

- Calculate separately for subgroups of women defined by:
 - 1) invitational status:
 - a. personally invited
 - b. not personally invited
 - c. unknown
 - 2) programme status, i.e., smear performed:
 - a. within organised programme
 - b. outside organised programme
 - c. unknown
- Stratification by 5-year age groups is also recommended.
- Obtain data from **Table B2** in annex of Chapter 2 (denominator and numerator).
- Also calculate separately with eligible women as denominator

N women screened
at least once in defined interval
(3 or 5 years)

N resident women in
target population

4. Compliance to invitation

- Consider women invited in a given period and those among them screened.
- A cut-off date of six months after the end of the respective period is recommended for determining whether a woman was screened in response to the invitation. If a different cut-off procedure is used, this should be specified.
- Obtain data from **Table B2** in annex of Chapter 2 (denominator and numerator).

$$\frac{\text{N invited women in a given period who were screened}}{\text{N invited women in that period}}$$
5. Smear consumption

- Include only screening smears (no repeat tests, e.g., after unsatisfactory smears or for follow-up) and count one test per 'screening episode'; see glossary.
- For denominator of a) see **Table B2**, annex to Chapter 2.

a)
$$\frac{\text{N screening tests in 3 (5) years in the target population}}{\text{N women in the target population screened in the same period}}$$

b) Distribution of screened women by number of screening smears in the same period.

6. Incidence of invasive cancer in unscreened and underscreened women in a given interval (3.5 or 5.5 years)

- Include only fully invasive cancer cases and person-years of the women not attending screening at the regular interval, i.e. women not screened in the previous 3.5 (5.5) years.
- Link screening registry and cancer registry data and calculate incidence age-adjusted, and by age group, based on the entire female population in the age groups eligible to attend screening.
- Analyse by cancer morphology (squamous vs. non-squamous)
- Calculate separately (with appropriate denominators):
 - women never screened
 - women previously screened, but interval to last screening test >3.5 (5.5) years
 - women never invited
 - invited vs. not invited in respective round

$$\frac{\text{N fully invasive cancers detected in women not screened in a given interval (3.5 or 5.5 years)}}{\text{N person-years of women not screened in the same interval (3.5 or 5.5 years)}}$$

7.5.2 Screening test performance

7. Distribution of screened women by the results of cytology

- Obtain data from **Table B3** (numerator) and **Table B2** (denominator) in annex to Chapter 2.
- Use classification in table B2 in annex to Chapter 2.
- Calculate overall and separately for subgroups of women:
 - a. for the regular screening interval and shorter time periods
 - b. attending initial or subsequent screening

N screened women with cytological diagnosis
N screened women

8. Referral rate for repeat cytology

- Obtain data from **Table B4** (numerator) and **Table B2** (denominator) in annex to Chapter 2.
- Calculate separately:
 - a. by cytology that resulted in recommendation to repeat
 - b. for initial and subsequent screening

N screened women advised to repeat test at shorter than regular interval
N screened women

9. Compliance with referral for repeat cytology

- See **footnote** in **Table B4** (numerator) and **Table B4** (denominator) in annex to Chapter 2.
- Calculate separately:
 - a. by cytology that resulted in recommendation to repeat
 - b. for initial and subsequent screening

N women screened following recommendation for repeat cytology
N women recommended for repeat cytology

10. Referral rate for colposcopy

- Obtain data from **Table B5** (numerator) and from **Table B2** (denominator) in annex to Chapter 2.
- Calculate separately by:
 - a. cytology that resulted in referral to colposcopy
 - b. for initial and subsequent screening

N screened women referred for colposcopy
N screened women

11. Positive predictive value of referral for colposcopy

- Obtain data from **Table B7** in annex to Chapter 2.
- If the number of women, for whom colposcopy was performed is not known, estimate using number of women referred for colposcopy.
- Calculate overall and separately by:
 - a. cytology (ASC-US+, LSIL+, HSIL+)
 - b. histology (CIN1+, CIN2+, CIN3+, Invasive Ca)
 - c. initial and subsequent screening

$$\frac{\text{N screened women who had colposcopy with histologically confirmed CIN+}}{\text{N screened women who had colposcopy}}$$
12. Test specificity

- Calculate overall, and separately by:
 - a. cytology (<ASC-US, <LSIL, <HSIL)
 - b. histology (CIN1+, CIN2+, CIN3+, Invasive Ca)
 - c. initial and subsequent screening
- Test specificity cannot be computed from routine screening and follow-up data, because the true denominator is unknown. Nevertheless, the formulas on the right should be used to approximate specificity.
- Normal test results refer to 'negative for intraepithelial lesions' (i.e., results not leading to referral for follow-up or confirmation)

$$\frac{\text{N screened women not referred for colposcopy}}{\text{N screened women who had no histologically confirmed CIN+}}$$

$$\frac{\text{N screened women with normal screening test results}}{\text{N screened women who had no histologically confirmed CIN+}}$$
13. Detection rate by histological diagnosis

- Obtain data from **Table B7** (numerator) and **Table B2** (denominator) in annex to Chapter 2.
- Calculate separately:
 - a. by histology (CIN1+, CIN2+, CIN3+, Invasive Ca)
 - b. for the regular screening interval and shorter time periods
 - c. for initial and subsequent screening

$$\frac{\text{N screened women with histologically confirmed CIN+}}{\text{N screened women}}$$

14. Cancer incidence after normal cytology

- Normal cytology refers to cases recommended for rescreening at the regular interval.
- Count only fully invasive cancers among the women who had a normal screening cytology in the previous 3.5 (5.5) years.
- Analyse by:
 - a. interval from index cytology
 - b. cancer morphology (squamous vs. non-squamous)
- Cytology should be reviewed mixed with that of other women not developing cancer.

$$\frac{\text{N screened women with fully invasive cervical cancer detected within 3.5 (5.5) years of normal cytology}}{\text{N person-years of screened women for same period after normal cytology}}$$
7.5.3 Diagnostic assessment and treatment**15. Compliance to referral for colposcopy**

- Obtain data from **Table B6** (denominator) and **Table B8** (numerator) in annex to Chapter 2.
- Calculate separately by:
 - a. different intervals after referral (3 months / 6 months)
 - b. cytology that resulted in referral

$$\frac{\text{N screened women actually undergoing colposcopy}}{\text{N screened women referred for colposcopy}}$$
16. Treatment of high-grade intraepithelial lesions

- Obtain data from Table B9 in annex to Chapter 2.

$$\frac{\text{N women with screen-detected CIN2 or CIN3 treated}}{\text{N women with screen-detected CIN2 or CIN3}}$$
17. Proportion (%) of women hysterectomised on screen-detected intraepithelial lesions

- Obtain data from Table B9 in annex to Chapter 2.
- Calculate separately by histology (CIN1, CIN2, CIN3).
- Appropriateness of individual cases should be evaluated by peer review.

$$\frac{\text{N screened women with histological CIN hysterectomised}}{\text{N screened women with histological CIN}}$$

18. Proportion (%) of women treated on CIN1

- Obtain data from **Table B9** in annex to Chapter 2.
- Appropriateness of individual cases should be evaluated by peer review.

N women with screen-detected CIN1 treated

N women with screen-detected CIN1

19. Incidence of invasive cancer after abnormal cytology

- Include screened women:
 - a. without colposcopy carried out, despite existing indication
 - b. with colposcopy carried out, but no CIN detected
 - c. with CIN detected, but not treated
 - d. treated
 - e. in diagnostic or post-treatment follow-up
- Calculate overall and separately for each of above subgroups.
- Include only fully invasive cancers.
- Exclude cases detected as a result of screening.

N cases of invasive cancer in screened women after abnormal cytology
--

N person-years of screened women after normal cytology
--

20. Proportion of women with cytology negative for SIL, 6 months after treatment

- Obtain data from **Table B10** in annex to Chapter 2.
- Include women treated for CIN2, CIN3, CGIN or AdenoCa in situ followed at least 6 months after treatment (denominator)
- Include women negative for hr-HPV (numerator), if this test is used for follow-up

N screened and treated women with negative cytology after 6 months
--

N screened and treated women followed-up for 6 months

Appendix 1

Guidance on communication with women and health professionals involved in cervical cancer screening

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1.1 Introduction

Screening differs from other health care activities, as it is usually a health professional that initiates the process with an apparently healthy individual. For this reason, the ethics of carrying out screening must be carefully considered. The screening process may be harmful or beneficial to the individual: there may be risks attached to the screening test or subsequent diagnostic tests, a false-positive result can cause unnecessary anxiety while a false-negative result can give false reassurance. Therefore, it is imperative to communicate in an appropriate and unbiased manner information about screening, mentioning both the hazards and the benefits of the screening procedures, to enable individuals to make an informed choice about attending screening. To achieve this, screening operators need to develop new and innovative information approaches based on understanding of the complexity of appropriate communication with individuals invited to attend screening.

The objective of this chapter is to give an insight into the issues of communicating information about screening and to provide some pragmatic suggestions on planning and developing written screening information tools.

The present appendix is an adaptation for cervical screening of a chapter in the recently published 4th edition of the "European guidelines on quality assurance in breast cancer screening and diagnosis" (Giordano L *et al.*, 2006). The manuscript was developed in collaboration with operators currently involved in running European breast and/or cervical screening programmes.

1.1.1 Issues relating to communicating information on cancer screening

1.1.1.1 Communicating information to enable decision-making

While the term 'information' refers to the mere transfer of data, communication is a more complex process. It implies that the person who receives the information can understand and make use of it. Communicating about health does not just include transmitting information. In order to communicate effectively and appropriately about health, it is important to be aware of the social and cultural factors that influence individuals' needs and behaviours. In addition, health communication has become more complex due to the exponential growth of scientific knowledge. This can generate confusion and lead to difficulties in the process of decision-making (Arkin, 1999).

It has been suggested that providing information to individuals with the purpose of helping them make choices and decisions requires new ways of interacting and communicating (Katz *et al.*, 1995). Relevant questions include: What background information must individuals receive? How deeply should health professionals probe for understanding? What constitutes irrelevant information that only tends to confuse? What words and explanations facilitate comprehension? Health professionals are not used to addressing such questions (Katz *et al.*, 1995).

Health professionals must provide individuals with information that will allow them to 'knowledgeably' decide whether or not to undergo an intervention, taking into consideration available alternatives, potential risks and foreseeable outcomes (Entwistle *et al.*, 1998; Goyder *et al.*, 2000). In the screening context, however, the issue of communication becomes more complex because in screening, it is the health professional (generally both administrative staff and medical personnel) who approaches an apparently healthy individual about undergoing a test. Women invited to have a screening test are not ill, and only a few of them will develop cervical cancer during

the course of their lives. It is therefore vital that these women know the pros and cons of screening to help them make an informed decision about whether or not to attend screening (Parker, 2001; Raffle, 2001; Coulter, 2001; Austoker, 1999). When a woman chooses to have a screening test, she voluntarily agrees to do so. However, this does not imply that she has knowledge and understanding of what is proposed (Slater, 2000).

The following factors have to be taken into account when developing communication strategies for women invited to attend screening.

1.1.1.2 Ethical principles

Any framework developed to communicate health information needs to be underpinned by the following ethical principles (Beauchamp & Childress, 1979):

- Autonomy: the obligation to respect the decision-making capacities of autonomous persons. This obligation emphasises that patients should normally be in a position to choose whether to accept an intervention or not as part of their general right to determine their own lives.
- Non-maleficence: the obligation to avoid causing harm intentionally or directly (the principle is not necessarily violated if a proper balance of benefits exists; that is, if the harm is not directly intended, but is an unfortunate side-effect of attempts to improve a person's health).
- Beneficence: the obligation to provide benefits, balancing them against risks.
- Justice: the obligation of fairness in the distribution of benefits and risks.

These four principles provide a useful framework for health professionals (including those offering screening) to use when developing appropriate ways of communicating with client groups.

1.1.1.3 Population heterogeneity and informed choice

There is a growing concern that individuals invited for screening are often told about the positive aspects of screening, ignoring any negative aspects in order to increase the attendance rate and ensure the effectiveness of the screening programme (Parker, 2001; Coulter, 1998; Baines, 2003). Women cannot be expected to make an informed choice about participation in a screening programme unless they are given sufficient and adequate information. This information should be honest, adequate, truthful, evidence based, accessible, unbiased, respectful, and tailored to individual needs (Goyder *et al.*, 2000; Austoker, 1999; Anderson & Nottingham, 1999; Raffle, 1997; Coulter *et al.*, 1999). Otherwise, problems could emerge. For example, misconceptions about cancer and the screening process may lead to high anxiety levels (Brett *et al.*, 1998).

In the screening context, the 'public' is not monolithic; instead, there is a diversity of 'publics', each having specific characteristics which need to be taken into account. Thus, while cervical cancer screening is a population programme, health professionals offering screening to the population have to deal with individuals of different ages and with different cultures, values and beliefs. For these reasons, the information provided may be viewed differently and what is best for one recipient may not be the best for another (Rimer *et al.*, 2004). In addition, contextual and personal factors may directly influence the way an individual processes health information and may therefore impact on the motivations to attend screening. Educational status can also have an impact on how the presented information is understood (Aro *et al.*, 1999; Lagerlund *et al.*, 2000b; Davis *et al.*, 2002).

1.1.1.4 The role of the media

An important factor that must be noted by health professionals is the influence of the mass media on individuals' perception and understanding of health issues. Research has shown that the media plays an important role in influencing opinion on the use of medical interventions such as screening (Passalacqua *et al.*, 2004). Generally, the media has favoured the optimistic message of the 'mythical' view that, medicine in general and screening, in particular, can cure or prevent all diseases. The information disseminated by the media has often underlined only benefits of medical services, glossing over uncertainties, adverse events and side-effects and ignoring legitimate scientific controversies (Grilli *et al.*, 2000; Dobias *et al.*, 2001; Wells *et al.*, 2001; Jorgensen & Gotzsche, 2004). With respect to screening programmes the message from the media appears to be that screening is 100% accurate and therefore any false positives or negatives must be due to errors on the part of those providing the screening. This has led to the perception that all cancers arising after a normal screening examination must have been 'missed'. This misunderstanding of the effectiveness of screening has resulted in high expectations on the part of the public and anger and resentment (sometimes resulting in litigation) when expectations are not fulfilled (Wilson, 2000).

Health professionals must therefore be aware of the role of the media in providing information and influencing individuals' decisions. It is important that persons in charge of the screening programme work closely with the media and provide them with current, accurate and comprehensive information proactively and regularly. Such information disseminated by the media may engender informed debate that empowers the public rather than giving rise to false expectations that cannot be realistically met by screening services.

1.1.2 Problems related to effective communication in screening

Problems of communication can be associated both with providers and consumers (Aro *et al.*, 1999; Lagerlund *et al.*, 2000; Theisen, 2004) as illustrated by the following key points.

1.1.2.1 Access to the information about cervical screening

Appropriate information in suitable formats should be available and accessible to all women who would benefit from cervical screening. It is important that women are informed about where they can get information about screening, what kind of information is available and in what format (written materials, web-sites, information phone lines, etc.). Accessibility also includes the provision of such information to disadvantaged groups (i.e. disabled, ethnic groups).

1.1.2.2 Screening knowledge and communication skills of health professionals involved in the screening programme

Women obtain information on cervical cancer from a variety of sources, among which health professionals are one of the most obvious. In most EU countries, general practitioners (GPs), gynaecologists and smear takers play a central role in the provision of this information.

GPs and gynaecologists' personal and continuing relationship with patients puts them in a privileged position for supplying these women with relevant and specific information. This can contribute to reducing anxiety and fear about the test. These professionals are also usually trusted by their patients, and overall their involvement in the decision-making process is accepted by women. Research indicates that their involvement is an important factor influencing screening coverage (Giorgi *et al.*, 2000; Clover *et al.*, 1996).

In the same way, smear takers are central Fig.s in optimising women's experience, satisfaction and the continued acceptance of screening. If they receive women in a calm, relaxed and friendly atmosphere, answering enquires and carefully explaining the procedures, they can generate confidence in women, and increase their co-operation, while minimising their anxiety.

If health professionals' involvement in communicating about cervical cancer screening is important, it follows that they need to acquire comprehensive knowledge, in order to give women adequate and accurate information about the pros and cons of screening and attendant processes. Educating, training and motivating health professionals to play an appropriate role in enabling and empowering women to make informed decisions about participation in cervical screening is a significant part of the proper running of a screening programme.

Unfortunately, biomedical ethics is rarely covered in the curriculum of the health operators of cervical screening programmes. Risk communication, i.e. communication of benefits, the potential harm from medical interventions, and the subtleties of what genuine informed consent involves are infrequent topics in medical and health education programmes.

The way in which information is presented is as important as the information itself. Accuracy of information depends not only on its content but also on the communication skills of the health professionals involved in providing it. Health professionals need to be sensitive to the educational, linguistic and religious differences among women. They should use jargon-free language and avoid incomprehensible mathematical or statistical concepts expressing risk (Doak *et al.*, 1998) as this makes it very difficult for lay people to understand what is being communicated. To overcome these problems, it is essential that health professionals are given appropriate training in communication skills.

A number of important tasks of health professionals are summarised in Table 1.

Table 1. Important issues in communication on screening by health professionals

1. General practitioners should complete screening and follow-up information in their patient's medical files.
2. General practitioners should discuss screening during contacts with female patients belonging to the target age group, in order to complete information lacking in their medical files and in order to motivate women to participate in (preferably organized) screening.
3. Gynaecologists, smear takers and/or cytological laboratories should ensure that GPs are informed of the screening status of their respective clients.
4. Laboratories (private or public) should give high priority to providing information to update the screening register.
5. Information on histology and follow-up or treatment needs should be reported back to cytological laboratories at which the respective screening smears were read.
6. General practitioners, gynaecologists, cyto-pathologists and other health professionals involved in cervical screening should receive feedback from national or regional statistical services.
7. Applicable laws and regulations on privacy protection and ethical rules must be followed in transfer of any personal medical data.

1.1.2.3 Consumers' health literacy skills and ethnical minorities

On the part of consumers, low health literacy skills can represent a major obstacle in understanding information. Individuals vary in their ability to read, understand and use information. Poverty, ethnicity and age are also considered predictors of limited literacy (Davis *et al.*, 2002). Providers of screening programmes frequently have to cater to multicultural and multi-linguistic populations with all the related communication problems. Overcoming these problems requires more than just translating the information material. Efforts must be made to gain an understanding of ethno-cultural values, beliefs, health practices and communication styles of these varied groups; and their specific information needs must be identified (van Wieringen *et al.*, 2002).

1.1.2.4 Developing client-centred information

Some phases of the screening process, especially the invitation phase, do not usually permit one-to-one communication between health professionals and individual clients. Transmission of information is frequently one-way, from the health professional and the information is often developed without any input from the client group, or without evaluation to determine its appropriateness to client needs. An important aspect of decision-making is that individuals have access to relevant and appropriate information, relevant not just from the health professional's point of view but also from the individual's point of view. It is therefore vital to ascertain the views of those invited for screening as to what information they require in order to make an informed choice and the communication process more effective and appropriate.

1.2 Suggestions on how to plan and develop screening information tools

As underlined in the previous pages of this appendix, women cannot make informed decisions unless they are provided with sufficient and appropriate information about cervical cancer and screening. The information women are given should be underpinned by available evidence and presented in an appropriate format.

1.2.1 Improving the quality of cervical cancer screening communication tools

Several tools have been used to convey information to individuals about screening interventions (videos, leaflets, audio cassettes and touch screens, etc.). A systematic review of informed use or non-use of screening concluded that "...there is currently limited evidence available about the most effective ways of presenting information about the risks and benefits of screening" (Jepson *et al.*, 2001).

The following questions should be asked when developing communication tools and information packages for cervix cancer screening (Entwistle *et al.*, 1998):

- Which is the most accessible media format?

- Can it be shared with family and friends?
- How easy would it be to update?
- What about the costs of updating, reproduction and distribution?
- Would it be appropriate and acceptable to the intended audience?

Key attributes of a good communication tool are summarized in Table 2.

Table 2. Attributes of a good communication tool

1. Easy to understand
2. Accessible and comprehensive
3. User-friendly
4. Easy and cost-effective to update, reproduce and distribute

Information provided to women invited to attend cervical cancer screening should be accessible, relevant, comprehensible, comprehensive; it should include benefits as well as risks and disadvantages, and it should be tailored to meet needs of special groups. In addition, information should be phase specific and multi-level, in order to take into account the needs of women with a positive test requiring further follow-up and/or treatment. These qualities are discussed briefly below.

Accessible

Information should be accessible to all women who would benefit from cervical screening. It is important that a woman who needs information about cervical screening should be able to find and access it easily.

Relevant

Screening information should be relevant to the women for whom it is intended. It should be 'women centred' and should meet their needs.

In the past, screening professionals tended to assume that they knew best what information women needed and wanted. Unfortunately the majority of the materials they developed failed to address issues that women thought important (Davey *et al.*, 2002). It is vital to gain an insight into women's understanding of the information about cervical screening and to involve them in developing information materials. However, this is rarely done. Screening providers should find out what information women need and want, and should involve women in the development of appropriate and timely information materials.

Comprehensible

Information should be clear, avoiding jargon and technical language.

Guidelines have been produced to enable the production of good written material (NHSCSP, 2006a; NHSCSP, 2006b; Albert & Chadwick, 1992). They include the following recommendations:

- Women's interest should be paramount.
- Use concepts the women will understand.
- Avoid unnecessary words.
- Be personal.
- Use short sentences and short words.
- Follow rules of grammar and syntax.

Comprehensive

Information should be comprehensive and messages should not be biased to encourage participation. It is imperative that the information is well balanced, i.e. it should include information on risks, false positives, false negatives, and uncertainties. Communication should also contain information about the benefits and the quality of the screening programme. It may be appropriate to add information about process indicators, such as participation rate, waiting times, proportion of inadequate and abnormal assessments, to help women understand the screening programme and to verify its results.

Tailored

Information should be tailored as far as is possible and adapted to suit the specific needs of different groups and different situations. This will ensure that the communication has more personal relevance and contains less redundant information. While it is difficult to provide individually tailored information for population programmes, it should be possible to provide particular sub-groups with relevant information tailored to their specific needs at a given stage of the screening process.

Phase-specific information

It would be appropriate to provide women with different types of information according to the different screening phases (i.e. first invitation, recall, etc.).

Research has shown a high level of anxiety experienced by women recalled for further assessment in cervical screening (Rogstad, 2002; French *et al.*, 2004; Bell *et al.*, 1995; Fylan, 1998; Wilkinson *et al.*, 1990; Marteau, 1989). This can be diminished, firstly, by informing women of the possibility of an inadequate or abnormal test requiring repetition and adequate follow-up, or even treatment at the appropriate time (Byrom *et al.*, 2003).

Information like 'what procedures are involved in further assessment' and 'the possible outcomes' should be given to women in the early phases of the screening process and, if necessary, repeated and expanded in subsequent phases using different formats.

Multi-level information

Basic information refers to the information handed out to all women (generally at the first invitation). It must be complete, honest and comprehensive and it should adhere to recommendations on readability and clarity (i.e., avoiding too much information, badly presented and using jargon that may lead to confusion). The basic information should be appropriate and brief, preferably in a question and answer format. However this format may limit the amount of information delivered, and women in the same screening phase may require different degrees of information, i.e. ranging from a basic level of information to more detailed information in specific areas. It is therefore important that women requiring additional and in-depth information are able to access it. Basic information provided to all women should also indicate where more detailed information may be obtained (i.e. phone-line, screening operators, GPs, web-sites, etc.). It is important that screening programmes use different communication instruments to provide this supplemental information.

Table 3 summarises the qualities which information provided to women invited to attend cancer screening should have.

Table 3. Qualities essential to information provided to women invited to attend cancer screening

Accessible:	Women should be able to find information easily.
Relevant:	Information should be “women-centred” and include items that women want to know.
Comprehensible:	Information should be clear, avoiding jargon and technical language.
Comprehensive:	Information should cover both the positive and negative aspects of screening.
Tailored:	Information should be customised to suit the specific needs of different groups and different situations.
Phase specific:	Women should be provided with information appropriate to the different screening phases (i.e., first invitation, follow-up, etc.)
Multi-level:	Information should be available from basic to more detailed information on specific aspects of screening and should be presented in different formats, in order to meet the needs of different users.

1.2.2 Recommendations on the contents of written information (invitation letter/leaflet)

The letter inviting women to participate in the screening programme is generally the first communication tool directly sent to women. It usually includes logistic/organisational information relating to the screening appointment.

Being the first contact with women, the invitation letter must be written in a simple, clear and readable style; it should include information about the purpose of the screening service. It is recommended that all relevant additional information is provided in a leaflet or other communication instruments sent with the letter. The letter should refer to the leaflet and encourage women to read it (Brett *et al.*, 1998). Table 4 lists the topics that should be covered in the invitation letter.

Invitation letters and leaflets are usually designed to complement each other and information contained in the former can be reiterated in the latter. The leaflet delivered with the invitation letter usually provides descriptive information about the screening programme, the test and its effects. It often reinforces information already mentioned in the invitation letter and adds extra information that may be useful to women.

The benefits and disadvantages of screening should be explained in the leaflet. It should be well written and visually acceptable to the audience. It is therefore important that different formats and structures are tested with the target population.

Table 5 lists the information that should be included in the leaflet.

Table 4. Contents of the invitation letter

Invitation letters should include information on (NHSCSP, 2006a; NHSCSP, 2006b):

1. The purpose of screening: who the test is for (target population - age group)
 2. Details of the screening test that will be performed.
 3. The screening interval
 4. If the test is free or not
 5. The appointment: how to make it, how to change it
 6. When and how to get the results (mentioning approximate waiting times)
 7. The possibility of having an abnormal or inadequate result (requiring follow-up)
 8. The validity of the test (having a Pap smear provides a low risk status, not a lack of risk of developing cervical cancer).
 9. Optional information: avoid vaginal douches/vaginal drugs <48 h before having the test, etc.
 10. Where women can obtain further information (e.g. information services, telephone hotlines, patient groups and web-sites).
 11. Data protection/confidentiality.
-

Table 5. Contents of the invitation leaflet

The invitation leaflet should include information about (NHSCSP, 2006a; NHSCSP, 2006b):

1. Who the test is for
 2. The test: nature, purpose, validity
 3. The process of the test: who performs the test, how long it takes, what it involves, how will it feel
 4. The screening interval (mentioning why the specified interval is used)
 5. What early detection means
 6. Benefits and disadvantages of cervical cancer screening (including information on side effects, i.e., the possibility of detecting lesions that usually regress, but which nevertheless require follow-up)
 7. How to obtain the result (approximate waiting times) and how to interpret it (negative, positive, uncertainties)
 8. Further assessment: explaining the possibility of further tests (why and what?) and the possibility of false positive results and uncertainties
 9. Quality control of the screening procedure
 10. Where women can obtain further information (e.g. information services, telephone hotlines, patient groups and web-sites)
 11. Date and sources of provided information
-

It is essential that written information is guided by good communication principles, as the way information is presented plays an important role in determining its comprehension and acceptance. Some recommendations on text and language style, wording, and formatting are provided in Table 6. They should be carefully considered by the screening staff to make the communication more effective and easily understandable to women.

Table 6. Stylistic advice

1. Language:

- Clear (about the topic: clarify points with examples)
- Honest, respectful, polite
- Simple everyday language (no technical terms, jargon, abbreviations and acronyms)
- Informal (use of pronouns like “we” and “you” to personalise the text)
- Impartial
- Not top-down (no prescriptive style or paternalistic tone)
- Written in the active voice.

2. Text style and wording:

- Credible, reliable (indicating the source of information)
- Up to date and contemporary
- Friendly and sympathetic
- Positively framed (e.g. 9 out of 10 recalled women are found to be normal rather than 1 out of 10 recalled women will have cancer)
- Positive tone (alarming statements should be avoided)

3. Text format:

- Preferably plain layout
 - Short sentences and brief paragraphs
 - Use of diagrams and pictures
 - Use of titles and subtitles (to distinguish different areas)
 - Bold or capital letters (to underline important points)
 - Larger print (essential for older target populations)
 - Use of white spaces (to facilitate reading)
 - Preferably question/answer and paragraph formats
 - Appropriate colours (as some colours are difficult for colour blind people to read)
 - Logo.
-

1.2.3 Other issues to consider when developing communication strategies for cervical screening

1.2.3.1 Relationship between information provision and participation in cervical cancer screening

It has been argued that the provision of explicit information on the limitations of screening could result in:

- decreased participation and reduction in population benefits;
- possible inequity, as those most likely to be deterred may be the most socially disadvantaged;
- increased costs as more staff time is required to explain screening and its consequences;
- reduced cost-effectiveness if participation falls so low that the service becomes unviable (Raffle, 2001).

There have been many debates in past years, about the desirability of attaining high rates of participation in screening “per se”, without allowing participants to make an informed decision about whether or not to be screened. As a result, tensions still exist between promoting informed decision-making, where the individual may decide not to undergo screening, and strategies promoting participation as an effective form of health-care (Austoker, 1999; Raffle, 1997; Jepson *et al.*, 2001; Thornton, 1995).

The concept of recognizing the active and responsible role of women and their participation in screening programmes, based on informed choice, has been proposed as a replacement of the idea of compliance (Segnan & Armaroli, 1999).

The question of how many people would refuse screening if the limitations were included in the information can be considered an empirical one, as very little work has been carried out in this area (Domenighetti *et al.*, 2000; Adab *et al.*, 2003). Research is needed to assess the impact of the “information factor” on participation.

1.2.3.2 The role of advocacy groups

The function of advocacy groups in cancer screening is increasingly essential (Ganz, 1995). We can refer, for example, to the role played by Europa Donna in the generation of breast awareness and lobbying for effective breast cancer screening programmes in Europe, and by the European Cervical Cancer Association (ECCA), established in 2002, to lead a pan-European public health education programme, bringing together cervical cancer specialists across Europe to focus on raising awareness of cervical cancer and means by which it can be prevented.

In recent years, advocacy groups have empowered women to evolve from the position of passive participants into influential partners (Avery & Bashir, 2003). The role of these and other such associations emphasises the importance and the need for screening and early detection, for defining and disseminating appropriate health education messages to the target audience, for gaining a better understanding of its informational requirements, for ensuring that women fully understand any proposed treatment options, and for providing high quality supportive care during and after treatment. Other aims of these associations are to advocate appropriate training for health professionals and to promote and support the advancement of cancer research and the dissemination and exchange of factual, up to date information on cancer.

1.2.3.3 The Internet

The advent of the Internet has added a new dimension in the dissemination of information and more people are turning to it to find health and cancer information (Satterlund *et al.*, 2003). Research indicates that higher usage of the Internet is associated with younger age, more education and higher income (Pereira *et al.*, 2000; Brodie *et al.*, 2001; Fox, 2000). Although the quality of medical information on the world wide web has been an area of increasing concern (Silberg *et al.*, 1997; Jadad & Gagliardi, 1998; Price & Hersh, 1999; Hoffman Goetz & Clarke, 2000), the factors that contribute to popularity of web-sites have not been systematically studied. For this reason, future studies should explore the use of this growing and increasingly accessible technology as a source of information.

1.2.3.4 Communication quality indicators

The development of indicators to evaluate the quality of the information provided to women in each screening programme should be an important aspect of the communication strategy in the future. Several technical indicators already exist to evaluate the performance of the screening procedure and the programme's activities. These have been incorporated into the quality assurance process of many ongoing screening programmes and are constantly reviewed and revised in the light of recent experience and research. In addition, minimum quality standards are recommended to evaluate programmes. However, no quality indicators are available to evaluate the standard of communication in screening. It is crucial that such standards are developed to assess the relevance and appropriateness of the information provided. In addition, indicators should be developed to assess how information about cervical screening is communicated to the women invited for screening and to the women in the different phases of screening or follow-up. Among the potential communication quality indicators, some lend themselves more to quantification, while others are more conceptual.

Table 7 outlines some indicators that could be refined and implemented to evaluate the quality of screening communication.

Table 7. Indicators for evaluating the quality of the screening communication

-
- The availability of a telephone information service for women invited for screening (YES/NO; number of calls received per 1,000 invited women)
 - The availability of different formats from which women can get information about the screening programme (YES/NO; types of formats)
 - Written information material which was tested on the target population for effectiveness, acceptability and readability (YES/NO; evaluation outcomes)
 - Information materials available for different ethnic groups or special needs groups (e.g. visually impaired) (YES/NO; proportion of specific communication materials for ethnic and/or disadvantaged minority groups compared to those present in the population)
 - The involvement of non-medical organisations (churches, stores, etc.) in the dissemination of information (YES/NO)
 - The implementation of counselling protocols (YES/NO; proportion of counselling sessions per 1,000 invited women)
 - The availability of face-to-face communication for women on request (YES/NO)
 - The availability of community volunteers and advocacy groups (YES/NO)
 - The organisation of courses on communication for screening providers (reception staff, smear takers, general practitioners, gynaecologists etc.) (YES/NO; number of courses/year; proportion of participants who took up the training compared to those who were eligible)
-

- Women's involvement in developing and assessing the information material (YES/NO)
- The administration of satisfaction questionnaires to the target population (YES/NO; evaluation outcomes)
- The availability of a web site (YES/NO; updating level, number of contacts).

1.2.3.5 Communication among screening operators

In addition to the bi-directional communication between health professionals and women, the efficiency of a successful screening programme also depends on the intercommunication within the team involved in the screening process, including screening providers and specialists involved in follow-up (colposcopy) and treatment; the feedback to health professionals and decision makers which is provided by the centre in charge of the statistical data analysis is also significant.

It would be beneficial to set up mutually supportive teams, understanding team relationships, defining roles and responsibilities, and sharing common experiences. At a more general level, effective co-operation and communication among health authorities, stakeholders, decision makers and health professionals, should also be established.

Good communication between all the sectors/interfaces involved in screening programmes is a factor crucial to the delivery of good quality communication and information to the target audience, helping to increase women's satisfaction and maximising the screening programme performance.

1.2.3.6 Information about HPV

With the establishment of the role of high-risk types of human papillomavirus (HPV) in the aetiology of most of cervical cancers, there is now widespread support for HPV testing in cervical cancer screening and the realistic expectation of immunisation against the virus through a vaccine (IARC, 1995). (More detailed information about cervical cancer and screening and HPV are reported in Table 8.)

Introducing HPV testing into cervical cancer screening will inevitably be accompanied by a shift in public understanding of the disease. Linking cervical cancer to a potentially stigmatising sexually transmitted infection could influence the psychological impact of abnormal screening results. It may also have implications for informed participation of women in HPV screening, and the likelihood of future participation (Waller *et al.*, 2005; McCaffery *et al.*, 2004; Waller *et al.*, 2003; McCaffery *et al.*, 2003). Many women do not know that cervical cancer is linked to sexual intercourse and may be shocked to discover that it is caused by a sexually transmitted virus.

Although there is limited literature on psychosocial reactions to HPV diagnosis, research among women who have received abnormal cervical cancer screening results indicates that they often experience psychological consequences, including anxiety, fears about cancer, relational problems, sexual difficulties, changes in body image and concerns about the loss of reproductive functions (Bell *et al.*, 1995; Maissi *et al.*, 2005; Lerman *et al.*, 1991; Campion *et al.*, 1988; Basen-Engquist *et al.*, 2003).

Psychological distress caused by the communication of a positive test result and the fears about gynaecological investigations and treatments have been shown to decrease compliance with follow-up recommendations. Therefore, counselling women has the potential to both enhance psychological well-being and improve follow-up and clinical outcomes. In the HPV scenario it becomes even more necessary to provide women with clear and consistent information about HPV to minimise anxiety and distress associated with uncertainty and confusion (French *et al.*, 2004; McCaffery *et al.*, 2004; Waller *et al.*, 2003; Mays *et al.*, 2000; Voog & Lowhagen, 1992; Monga *et al.*, 1997).

Health professionals and screening operators must develop educational strategies to explain HPV, addressing the sexually transmitted nature of HPV, the natural history and outcomes of HPV infection, the medical nomenclature encompassing HPV and where HPV testing fits within current cervical cancer screening guidelines (Harper, 2004).

The means by which information on this issue can be conveyed also need to be accurately identified. Web-sites or leaflets targeting women with abnormalities for whom HPV triage is recommended, should be planned to provide more detailed information about HPV infection. Invitation letters do not seem adequate to deliver this information.

Considering the complexity of this type of communication, tailored HPV information related to women's background characteristics, should take into account mainly age, type of HPV detected and women's literacy level (Anhang *et al.*, 2004ab).

For example, younger women have expressed more interest in information about HPV detection, infection and transmission, and its role in the development of cervical cancer. Women have also expressed preferences for HPV education that is tailored to the low or high-risk strains identified by HPV testing (Goldsmith *et al.*, 2007; Anhang *et al.*, 2004a; Anhang *et al.*, 2004b).

Furthermore, women may find it difficult to deal with HPV testing information, due to sometimes insufficient or inaccurate health knowledge (Davis *et al.*, 2002), poor numeracy skills and impaired ability to assimilate new information. In these instances, health professionals should provide adequate information about screening options in clear, face-to-face conversations. For such activities, health professionals will require appropriate training and awareness of the need to avoid generation of psycho-relational problems (French *et al.*, 2004; Maissi *et al.*, 2005; Harper, 2004).

Particular attention must be paid to the information needs of women subject to other disparities in cervical cancer screening, such as women of low income living in rural areas, older women and immigrants (Marrett *et al.*, 2002; Ajayi & Adewole, 1998).

Finally, salient and accurate media information will also be of great importance in efforts to inform women about screening choices and to manage psychosocial responses to HPV DNA test results. Media coverage of HPV should respond to women's educational needs by including information about low-risk and high-risk types of HPV and differences in their significance for cervical cancer, which can help to explain benefits and consequences of HPV testing (Anhang *et al.*, 2004a).

In dealing with HPV, the challenge is to develop ways of communicating accurate information about benefits and the associated risks within cervical screening initiatives such that women understand both the prevention and management issues associated with the virus.

1.3 Developing a communication strategy for cervical cancer screening – a summary

The communication strategy for cervical cancer screening must be underpinned by robust ethical principles and ensure that the information developed is evidence-based, 'women centred' and delivered effectively.

Screening providers should therefore consider the following key points when planning and developing communication strategies for cervical screening.

- Take into account the principles of bioethics (autonomy, non- maleficence, beneficence, justice).
- Have conclusive evidence that screening procedures meet the appropriate criteria and can be of potential benefit to individuals (Committee of Ministers, 1994).
- Accept and involve women as dynamic partners.
- Provide individuals with information that will allow them to make an informed choice.
- Acquire the comprehensive knowledge needed to inform people about the pros and cons of screening.
- Be sensitive to educational, linguistic, cultural and religious differences among individuals and tailor information to suit personal needs.
- Explore women's information needs and involve them in developing information materials.
- Take into account the needs of disadvantaged groups (disabled, ethnic minority groups, visually impaired, etc.).
- Give appropriate information in suitable formats available and accessible to the target population.
- Test the different information aids on a sample of the target population to evaluate their effectiveness.
- Evaluate women's satisfaction with the screening service (by surveys or questionnaires).
- Develop standards to evaluate the quality of the provided information.
- Give women opportunities to discuss the options available, if a test appears to be positive, with screening professionals in a supportive environment.
- Avoid situations where economic or political incentives could affect the messages.
- Involve GPs in screening programmes, as women usually know them and tend to have a good relationship with them.
- Enhance and improve communication within all the sectors/interfaces involved in the screening programme (from health authorities, stakeholders and decision makers to health professionals and screening operators).
- Collaborate with advocacy groups.
- Collaborate with the media to ensure the dissemination of accurate information on cervical cancer screening.
- Co-ordinate and collaborate with other credible sources.
- Exploit new communication tools (Internet, videos, touch-screen computers).
- Reserve funds and personnel dedicated to communication.
- Receive adequate and on-going training in communication skills.

- In the case of HPV testing, pay particular attention to appropriately inform women about the sexually transmitted nature of HPV, the natural history and outcomes of HPV infection and its role in development of cervical cancer.

1.2.4 ECCA Key messages

ECCA (European Cervical Cancer Association) has identified some key messages and advice related to cervical cancer screening and HPV that could be useful in developing cervical screening information material, together with the other recommendations previously discussed in this chapter.

Table 8. ECCA key messages about cervical cancer screening and HPV

Cervical cancer key Fig.s

- Each year about 60,000 women in Europe develop cervical cancer and almost 30,000 women die from this disease. The majority of cases occur in women who have not been regularly screened. Where screening using Pap smears was well organised, incidence of and mortality from cervical cancer dropped dramatically.

Cervical cancer development

- Cervical cancer is believed to take a long time to develop, perhaps 10-15 years.
- Cervical cancer only develops when a HPV infection is not cleared and remains for many years.

If your immune system clears the virus, the risk of developing cervical cancer returns to normal.

Cervical screening

Cervical screening helps to prevent cervical cancer by finding early cervical cell abnormalities so that they can be treated before this cancer can develop.

- The earlier the cervical cell abnormalities are detected, the easier they are to treat and the more successful the treatment will be.
- Cervical screening, based on the Pap test, can, if well organised, result in 80% reduction of cervical cancer incidence and mortality.
- Cervical screening offers the best protection if you are screened regularly.

Most women with an abnormal Pap smear result will not require treatment, but a few women will have higher-grade cervical cell abnormalities that do need treatment. That is why it is extremely important that all abnormal test results are followed-up appropriately.

Treatment of abnormal cervical cells

- Follow-up of your screening test may find higher-grade abnormalities that require treatment. These treatments can usually be done in the outpatient clinic using procedures that have a very high degree of success.

Human Papillomavirus (HPV) – as the cause of cervical cancer

- There are many types of HPV; some cause skin warts, some cause genital warts and some can cause cervical cancer.

- More than half of the women who get HPV will clear the infection in 6-12 months without any treatment.
- Smoking appears to delay or prevent clearing of HPV.
- HPV infections can cause abnormal cervical cells on your Pap smear. Once the virus has been cleared, the abnormal cells will also usually disappear.
- Only infections that are not cleared give any risk for the future development of cervical cancer, but having been identified, the risk can be managed and reduced by regular screening.
- There is no treatment for HPV infection, but most infections clear by themselves. If the virus does not clear and causes abnormal cervical cells, treatment of these usually clears the HPV as well.

Human Papillomavirus (HPV) – as a sexually transmitted infection

- HPV is very common: 8 out of every 10 sexually active adults having had an infection at some time in their lives.
- Condoms are not fully protective against HPV infection but do protect against other sexually transmitted infections.
- HPV infections do not produce symptoms: you can have an infection and never know about it.
- HPV infections can remain – without any symptoms – for many years and it is impossible to know when you got the infection.
- There is no treatment for HPV infection but most infections disappear on their own.
- There is not a reliable technique for testing men and a negative result in a man may not be correct.

HPV testing

HPV testing has been proposed for three possible uses in cervical cancer prevention:

- As a screening test together with the Pap smear. Because HPV infection is very common in younger women, the use of HPV testing to screen women under the age of 30 is not recommended. Large screening trials are currently being conducted to verify if screening using HPV testing alone or in combination with a smear is better than with the Pap smear alone. Within a few years it will be possible give more clear answers to this question.
- Currently, there is evidence that HPV testing is useful for the follow-up of women with atypical Pap smears to identify those who may need treatment.
- Also for the follow-up of women who have been treated for cervical cell abnormalities, HPV testing is useful to monitor success or failure of treatment.

HPV vaccination

Vaccines to prevent HPV infection have shown very good results in clinical trials. If these trials continue to go well, it is likely that a vaccine against HPV will be available within the next 2 years. Nevertheless, HPV vaccination essentially will protect people not yet infected. The vaccine will therefore be offered to girls before the onset of sexual activity. It will take decades before incidence from cervical cancer will fall as a consequence of vaccination. Thus priority should be given to the organisation of screening until at least 2040.

1.4 References

- Adab P., Marshall T., Rouse A., Randhawa B., Sangha H., & Bhangoo N. (2003). Randomised controlled trial of the effect of evidence based information on women's willingness to participate in cervical cancer screening. *J. Epidemiol. Community Health* **57**: 589-593.
- Ajayi I.O. & Adewole I.F. (1998). Knowledge and attitude of general outpatient attendants in Nigeria to cervical cancer. *Cent. Afr. J. Med.* **44**: 41-43.
- Albert T. & Chadwick S. (1992). How readable are practice leaflets? *BMJ* **305**: 1266-1268.
- Anderson C.M. & Nottingham J. (1999). Bridging the knowledge gap and communicating uncertainties for informed consent in cervical cytology screening; we need unbiased information and a culture change. *Cytopathology* **10**: 221-228.
- Anhang R., Goodman A., & Goldie S.J. (2004a). HPV communication: review of existing research and recommendations for patient education. *CA Cancer J. Clin.* **54**: 248-259.
- Anhang R., Wright T.C., Smock L., & Goldie S.J. (2004b). Women's desired information about human papillomavirus. *Cancer* **100**: 315-320.
- Arkin E.B. (1999). Cancer risk communication-what we know. *J. Natl. Cancer Inst. Monogr* 182-185.
- Aro A.R., de Koning H.J., Absetz P., & Schreck M. (1999). Psychosocial predictors of first attendance for organised mammography screening. *J. Med. Screen.* **6**: 82-88.
- Austoker J. (1999). Gaining informed consent for screening. Is difficult--but many misconceptions need to be undone. *BMJ* **319**: 722-723.
- Avery B. & Bashir S. (2003). The road to advocacy--searching for the rainbow. *Am. J. Public Health* **93**: 1207-1210.
- Baines C.J. (2003). Mammography Screening: Are Women Really Giving Informed Consent? *JNCI Cancer Spectrum* **95**: 1508-1511.
- Basen-Engquist K., Paskett E.D., Buzaglo J., Miller S.M., Schover L., Wenzel L.B., & Bodurka D.C. (2003). Cervical cancer. *Cancer* **98**: 2009-2014.
- Beauchamp T.L. & Childress G. (2001). Principles of biomedical ethics. New York: Oxford University Press, 1st ed, 1979; 5th ed.
- Bell S., Porter M., Kitchener H., Fraser C., Fisher P., & Mann E. (1995). Psychological response to cervical screening. *Prev. Med.* **24**: 610-616.
- Brett J., Austoker J., & Ong G. (1998). Do women who undergo further investigation for breast screening suffer adverse psychological consequences? A multi-centre follow-up study comparing different breast screening result groups five months after their last breast screening appointment. *J. Public Health Med.* **20**: 396-403.
- Brodie M., Foehr U., Rideout V., Baer N., Miller C., Flournoy R., & Altman D. (2001). Communicating health information through the entertainment media. *Health Aff. (Millwood.)* **20**: 192-199.
- Byrom J., Dunn P.D., Hughes G.M., Lockett J., Johnson A., Neale J., & Redman C.W. (2003). Colposcopy information leaflets: what women want to know and when they want to receive this information. *J. Med. Screen.* **10**: 143-147.

- Campion M.J., Brown J.R., McCance D.J., Atia W., Edwards R., Cuzick J., & Singer A. (1988). Psychosexual trauma of an abnormal cervical smear. *Br J Obstet Gynaecol* **95**: 175-181.
- Clover K., Redman S., Forbes J., Sanson-Fisher R., & Callaghan T. (1996). Two sequential randomized trials of community participation to recruit women for mammographic screening. *Prev Med* **25**: 126-134.
- Committee of Ministers. Council of the Europe Recommendations n° R (1994). 11 of the Committee of Ministers to member states on screening as a tool of preventive medicine 94.
- Coulter A. (1998). Evidence based patient information. *BMJ* **317**: 225-226.
- Coulter A. (2001.) Patient-centered decision making: empowering women to make informed choices. *Womens Health Issues* **11**: 325-330.
- Coulter A., Entwistle V., & Gilbert D. (1999). Sharing decisions with patients: is the information good enough? *BMJ* **318**: 318-322.
- Davey H.M., Barratt A.L., Davey E., Butow P.N., Redman S., Houssami N., & Salkeld G.P. (2002). Medical tests: women's reported and preferred decision-making roles and preferences for information on benefits, side-effects and false results. *Health Expect* **5**: 330-340.
- Davis T.C., Williams M.V., Marin E., Parker R.M., & Glass J. (2002). Health literacy and cancer communication. *CA Cancer J. Clin.* **52**: 134-149.
- Doak C.C., Doak L.G., Friedell G.H., & Meade C.D. (1998). Improving comprehension for cancer patients with low literacy skills: strategies for clinicians. *CA Cancer J. Clin.* **48**: 151-162.
- Dobias K.S., Moyer C.A., McAchran S.E., Katz S.J., & Sonnad S.S. (2001). Mammography messages in popular media: implications for patient expectations and shared clinical decision-making. *Health Expect* **4**: 127-135.
- Domenighetti G., Grilli R., & Maggi J.R. (2000). Does provision of an evidence-based information change public willingness to accept screening tests? *Health Expect* **3**: 145-150.
- Entwistle V.A., Sheldon T.A., Sowden A., & Watt I.S. (1998). Evidence-informed patient choice. Practical issues of involving patients in decisions about health care technologies. *Int. J. Technol. Assess. Health Care* **14**: 212-225.
- Fox S. (2000). The on line health care revolution: how the Web helps Americans take better care of themselves. Washington, DC, The Pew Internet & American Life Project 2000.
- French D.P., Maissi E., & Marteau T.M. (2004). Psychological costs of inadequate cervical smear test results. *Brit J Cancer* **91**: 1887-1892.
- Fylan F. (1998). Screening for cervical cancer: a review of women's attitudes, knowledge, and behaviour. *Br. J. Gen. Pract.* **48**: 1509-1514.
- Ganz P.A. (1995). Advocating for the woman with breast cancer. *CA Cancer J. Clin.* **45**: 114-126.
- Giordano L, Webster P, Segnan N, Austoker J (2006). Chapter 12. Guidance on breast screening communication. in: European Commission (corporate author). European guidelines for quality assurance in breast cancer screening and diagnosis. Fourth edition. Perry N, Broeders M, de Wolf C, Törnberg S, Holland R., von Karsa L, Puthaar E (eds). Office for Official Publications of the European Communities, Luxembourg, pp 379-394.

Giorgi D., Giordano L., Senore C., Merlino G., Negri R., Cancian M., Lerda M., Segnan N., & Del Turco M.R. (2000). General practitioners and mammographic screening uptake: influence of different modalities of general practitioner participation. *Working Group. Tumori* **86**: 124-129.

Goldsmith M., Bankhead CR., Kehoe ST., Marsh G., Austoker J. (2007). Information and cervical screening: A qualitative study of English women's awareness, understanding and information needs about HPV. *J. Med. Screen*; **14**(1):29-33.

Goyder E., Barratt A., & Irwig L.M. (2000). Telling people about screening programmes and screening test results: how can we do it better? *J. Med. Screen.* **7**: 123-126.

Grilli R., Freemantle N., Minozzi S., Domenighetti G., & Finer D. (2000). Mass media interventions: effects on health services utilisation. *Cochrane Database Syst. Rev.* CD000389.

Harper D.M. (2004). Why am I scared of HPV? *CA Cancer J Clin* **54**: 245-247.

Hoffman Goetz L. & Clarke J.N. (2000). Quality of Breast Cancer Sites on the World Wide Web. *Can J Public Health* **91**: 281-284.

IARC (1995). Monographs on the evaluation of cancerogenic risks to humans. 35-86.

Jadad A.R. & Gagliardi A. (1998). Rating health information on the Internet: navigating to knowledge or to Babel? *JAMA* **279**: 611-614.

Jepson R.G., Forbes C.A., Sowden A.J., & Lewis R.A. (2001). Increasing informed uptake and non-uptake of screening: evidence from a systematic review. *Health Expect.* **4**: 116-126.

Jorgensen K.J. & Gotzsche P.C. (2004). Presentation on websites of possible benefits and harms from screening for breast cancer: cross sectional study. *BMJ* **328**: 148.

Katz J., Arras J.D., & Steinbock B. (1995). Informed consent: ethical and legal issues. *Anonymous Ethical Issues in Modern Medicine* 87-96.

Lagerlund M., Sparen P., Thurfjell E., Ekblom A., & Lambe M. (2000). Predictors of non-attendance in a population-based mammography screening programme; socio-demographic factors and aspects of health behaviour. *Eur. J. Cancer Prev.* **9**: 25-33.

Lerman C., Miller S.M., Scarborough R., Hanjani P., Nolte S., & Smith D. (1991). Adverse psychologic consequences of positive cytologic cervical screening. *Am. J. Obstet. Gynecol.* **165**: 658-62.

Maissi E., Marteau T.M., Hankins M., Moss S., Legood R., & Gray A. (2005). The psychological impact of human papillomavirus testing in women with borderline or mildly dyskaryotic cervical smear test results: 6-month follow-up. *Brit J Cancer* **92**: 990-994.

Marrett L.D., Robles S., Ashbury F.D., Green B., Goel V., & Luciani S. (2002). A proposal for cervical screening information systems in developing countries. *Int. J. Cancer* **102**: 293-299.

Marteau T.M. (1989). Psychological costs of screening. *BMJ* **299**: 527.

Mays R.M., Zimet G.D., Winston Y., Kee R., Dickes J., & Su L. (2000). Human papillomavirus, genital warts, Pap smears, and cervical cancer: knowledge and beliefs of adolescent and adult women. *Health Care Woman Int* **21**: 361-374.

McCaffery K., Forrest S., Waller J., Desai M., Szarewski A., & Wardle J. (2003). Attitudes towards HPV testing: a qualitative study of beliefs among Indian, Pakistani, African-Caribbean and white British women in the UK. *Brit J Cancer* **88**: 42-46.

- McCaffery K., Waller J., Forrest S., Cadman L., Szarewski A., & Wardle J. (2004). Testing positive for human papillomavirus in routine cervical screening: examination of psychosocial impact. *BJOG* **111**: 1437-1443.
- Monga U., Tan G., Ostermann H.J., & Monga T.N. (1997). Sexuality in head and neck cancer patients. *Arch. Phys. Med. Rehabil.* **78**: 298-304.
- NHSCSP. Improving the quality of the written information sent to women about cervical screening: Evidence-based criteria for the content of letters and leaflets. Goldsmith M., Bankhead C., Austoker J. NHSCSP (20006a). Publication N° 26. Sheffield: NHS Cancer Screening Programmes.
- NHSCSP. Improving the quality of the written information sent to women about cervical screening. Guidelines on the content of letters and leaflets. Goldsmith M., Bankhead C., Austoker J. NHSCSP (2006b). Publication N° 27. Sheffield: NHS Cancer Screening Programmes.
- Parker M. (2001a). The ethics of evidence-based patient choice. *Health Expect.* **4**: 87-91.
- Passalacqua R., Caminiti C., Salvagni S., Barni S., Beretta G.D., Carlini P., Contu A., Di Costanzo F., Toscano L., & Campione F. (2004). Effects of media information on cancer patients' opinions, feelings, decision-making process and physician-patient communication. *Cancer* **100**: 1077-1084.
- Pereira J.L., Koski S., Hanson J., Bruera E.D., & Mackey J.R. (2000). Internet usage among women with breast cancer: an exploratory study. *Clin. Breast Cancer* **1**: 148-153.
- Price S.L. & Hersh W.R. (1999). Filtering Web pages for quality indicators: an empirical approach to finding high quality consumer health information on the World Wide Web. *Proc.AMIA.Symp.* 911-915.
- Raffle A.E. (1997). Informed participation in screening is essential. *BMJ* **314**, 1762-1763.
- Raffle A.E. (2001a). Information about screening - is it to achieve high uptake or to ensure informed choice? *Health Expect.* **4**: 92-98.
- Rimer B.K., Briss P.A., Zeller P.K., Chan E.C., & Woolf S.H. (2004). Informed decision making: what is its role in cancer screening? *Cancer* **101**: 1214-1228.
- Rogstad K.E. (2002). The psychological impact of abnormal cytology and colposcopy. *BJOG* **109**: 364-368.
- Satterlund M.J., McCaul K.D., & Sandgren A.K. (2003). Information gathering over time by breast cancer patients. *J. Med. Internet.Res.* **5**: e15.
- Segnan N. & Armaroli P. (1999). Compliance, conscious participation, and informed consent in tumor screening programs. *Epidemiol Rev* **23**(4): 387-391.
- Silberg W.M., Lundberg G.D., & Musacchio R.A. (1997). Assessing, controlling, and assuring the quality of medical information on the Internet: Caveant lector et viewor--Let the reader and viewer beware. *JAMA* **277**: 1244-1245.
- Slater D.N. (2000). Are women sufficiently well informed to provide valid consent for the cervical smear test? *Cytopathology* **11**: 166-170.
- Theisen C. (2004). In different cultures cancer screening presents challenges. *J. Natl. Cancer Inst.* **96**: 10-12.
- Thornton H. (1995). Screening for breast cancer. Recommendations are costly and short sighted. *BMJ* **310**: 1002.

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van Wieringen J.C., Harmsen J.A., & Bruijnzeels M.A. (2002). Intercultural communication in general practice. *Eur. J. Public Health* **12**: 63-68.

Voog E. & Lowhagen G.B. (1992). Follow-up of men with genital papilloma virus infection. Psychosexual aspects. *Acta Derm. Venereol.* **72**: 185-186.

Waller J., McCaffery K., Forrest S., Szarewski A., Cadman L., & Wardle J. (2003). Awareness of human papillomavirus among women attending a well woman clinic. *Sex. Transm. Infect.* **79**: 320-322.

Waller J., McCaffery K., Nazroo J., & Wardle J. (2005). Making sense of information about HPV in cervical screening: a qualitative study. *Brit J Cancer* **92**: 265-270.

Wells J., Marshall P., Crawley B., & Dickersin K. (2001). Newspaper reporting of screening mammography. *Ann. Intern. Med.* **135**: 1029-1037.

Wilkinson C., Jones J.M., & McBride J. (1990). Anxiety caused by abnormal result of cervical smear test: a controlled trial. *BMJ* **300**: 440.

Wilson R.M. (2000). Screening for breast and cervical cancer as a common cause for litigation. *BMJ* **320**: 1352-1353.

Appendix 2

HPV vaccination – An overview

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As a matter of editorial policy, the second edition of the European Guidelines for Quality Assurance in Cervical Cancer Screening provides recommendations on prevention of cervical cancer through early detection programmes for cervical lesions. An appendix on prophylactic HPV vaccination has been added to the second guideline edition in order to summarise current evidence in this area. None of the statements in the appendix on HPV vaccination have the status of European Guideline recommendations on vaccination policies.

Development of comprehensive guidelines on prevention of cervical cancer which appropriately integrate screening and vaccination strategies is a key aim of the next phase of project activities supported by the EU Public Health Programme.

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This appendix consists of two parts:

A previously published article (Arbyn M., Dillner J. (2007). *Review of current knowledge on HPV vaccination. An appendix to the European Guidelines for Quality Assurance in Cervical Cancer Screening, J Clin Virology*. **38**:189-197) which is reproduced with permission from Elsevier, and an addendum with an update on evidence which has become available since preparation of the previously published manuscript.

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Review

Review of current knowledge on HPV vaccination: An Appendix to the European Guidelines for Quality Assurance in Cervical Cancer Screening

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Abstract

The recognition of a strong etiological relationship between infection with high-risk human papillomaviruses and cervical cancer has prompted research to develop and evaluate prophylactic and therapeutic vaccines. One prophylactic quadrivalent vaccine using L1 virus-like particles (VLP) of HPV 6, 11, 16 and 18 is available on the European market since the end of 2006 and it is expected that a second bivalent vaccine containing VLPs of HPV16 and HPV18 will become available in 2007. Each year, HPV16 and HPV18 cause approximately 43,000 cases of cervical cancer in the European continent. Results from the phase-IIb and III trials published thus far indicate that the L1 VLP HPV vaccine is safe and well-tolerated. It offers HPV-naïve women a very high level of protection against HPV persistent infection and cervical intra-epithelial lesions associated with the types included in the vaccine. HPV vaccination should be offered to girls before onset of sexual activity.

While prophylactic vaccination is likely to provide important future health gains, cervical screening will need to be continued for the whole generation of women that is already infected with the HPV types included in the vaccine. Phase IV studies are needed to demonstrate protection against cervical cancer and to verify duration of protection, occurrence of replacement by non-vaccine types and to define future policies for screening of vaccinated cohorts.

The European Guidelines on Quality Assurance for Cervical Cancer Screening provides guidance for secondary prevention by detection and management of precursors lesions of the cervix. The purpose of the appendix on vaccination is to present current knowledge. Developing guidelines for future use of HPV vaccines in Europe, is the object of a new grant offered by the European Commission.

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Keywords: HPV; Vaccine; Guideline; Cervical cancer

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1. Introduction

The current review is an appendix to the European Guidelines on Quality Assurance in Cervical Cancer Screening, which will be published early 2007 and where guidance is provided on organised secondary prevention in the member states of the European Union. The European Commission is currently preparing a grant to develop supplementary recommendations on future use of HPV vaccination.

Persistent infection of the uterine cervical epithelium with oncogenic human papillomavirus types is a necessary but insufficient causal factor in the carcinogenesis of cervical cancer (Bosch et al., 2002). The recognition of this strong causal association had led to the development of several prototypes of prophylactic and therapeutic vaccines (Frazer, 2004; Galloway, 2003; Schneider and Gissmann, 2003; Tjalma et al., 2004). Recently, an IARC expert group confirmed that for thirteen HPV types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66) there is sufficient evidence that they can cause cervical cancer (Cogliano et al., 2005; IARC, 2007). Pooled case-control studies indicate possible involvement of five additional types (HPV26, 53, 68, 73 and 82) in cervical carcinogenesis (Munoz et al., 2003, 2006). Moreover, HPV16 and (to a lesser degree) HPV18 are linked with more rare cancers, namely cancer of the vulva and vagina in women, cancer of the penis in men, and cancer of the anus, oropharynx and larynx in women and men (Parkin and Bray, 2006).

In this review, we briefly address some immunologic aspects of HPV infection and summarize the published results of placebo-controlled phases-II and -III vaccination trials. Some relevant public health questions concerning future prophylactic and therapeutic immunisation are also discussed.

2. Immunity against human papillomaviruses

2.1. Humoral immunity

HPV infection is restricted to epithelial cells; therefore presentation of viral antigens to the host immune sys-

tem is limited. Natural HPV infection of the genital tract gives rise to a slow and modest but measurable serum antibody response in most but not all infected individuals (Carter et al., 1996, 2000). The intensity of this humoral response depends on viral load and persistence (Ho et al., 2004). The presence of HPV antibodies is long lasting but does not contribute to the clearance of established infections (Shah et al., 1997). HPV serology is an important tool in epidemiological studies to assess past exposure (Dillner, 1999; Dillner et al., 1996, 1997; Lehtinen et al., 2001).

The capsid of papillomaviruses is composed of two viral proteins: the major capsid protein, or L1, and the minor capsid protein, or L2 (Orth and Favre, 1985). Virus-neutralising anti-L1 antibodies are generated against epitopes at the surface of the viral capsid and are essentially type-specific (Carter et al., 2000; Hines et al., 1994; Roden et al., 1996). The L2 protein is situated more internally of the capsid, but a small segment is exposed at the surface, and this segment can induce virus-neutralising antibodies as well (Christensen et al., 1991; Kawana et al., 1999; Roden et al., 2000). These anti L2-antibodies are less potent than anti-L1 antibodies (Christensen et al., 1991; Roden et al., 2000; White et al., 1999) but they appear to show some cross-reactivity to heterologous HPV types (Greenstone and Nieland, 1998; Nieland and Da Silva, 1999).

There is a series of methodological issues that make it difficult to unambiguously study whether immunity against type-specific reinfection occurs. Significant, though not complete, protection against reinfection has been found to be associated with the presence of HPV antibodies (Konya and Dillner, 2001). Other studies have shown that antibodies elicited by natural infection with a specific HPV type does not confer protection, since sero-positivity is not significantly associated with reduction in re-infection with homologous types (Viscidi et al., 2004).

The discovery that the L1 capsid protein could be expressed in eukaryotic cells and could self assemble into so-called virus-like particles (VLPs) was a critical step in the development of HPV vaccines (Zhou et al., 1991). HPV

L1 VLPs contain the same conformationally dependent neutralizing epitopes that are present on infectious viruses. The structural integrity of capsid proteins is necessary to elicit protective antibodies (Kimbauer et al., 1994). Denaturation or improper folding of the L1 protein alters the presentation of epitopes and yield unprotective antibodies. The L2 protein can also be expressed with L1 protein in yeast or insect cells, giving rise to “L1 plus L2”.

2.2. Cellular immunity

Clearance of a naturally acquired HPV infection is triggered by a specific cell-mediated immune (CMI) response. This subject was extensively reviewed by Man (1998). Dendritic cells or Langerhans cells, present in the cervical epithelium, play an important role in recognizing HPV infected cells and stimulating Th1 helper cells, which elicits the production of cytotoxic T-lymphocytes (CTL) (Niedergang et al., 2004). These cytotoxic effector cells attack infected cells, resulting in the resolution of the infection (Stern, 2004). However, little is known about how to modulate these immune responses.

3. HPV vaccination

3.1. Prophylactic vaccination

Vaccination with VLPs gives rise to virus-neutralizing antibodies in serum. Vaccination by intramuscular injection of L1 VLPs has been shown to be highly immunogenic and well tolerated in phase-I trials. Recently, three randomised placebo-controlled phase-II trials with, respectively, a monovalent HPV16 vaccine, a bivalent HV16/18 vaccine and a quadrivalent HPV6/11/16/18 vaccine candidate have consistently demonstrated almost complete protection against persistent infection with the targeted HPV types (Harper et al., 2004, 2006; Koutsky et al., 2002; Mao et al., 2006; Villa et al., 2005). Moreover, these trials confirmed the safety of the vaccines and showed strong immuno-responses that were several orders of magnitude higher than those observed after natural infections. All the trials showed 100% protection against the development of CIN associated with the HPV types included in the vaccines, although the trials were insufficiently powered to prove this hypothesis. The characteristics and main reported results of these studies are summarized in Table 1.

Two pharmaceutical companies (Merck Sharp and Dohme [MSD] and GlaxoSmithKline [GSK]) are currently conducting large multi-centre phase-III vaccine trials in all continents except Africa (Table 2) (Cohen, 2005). In addition, the National Cancer Institute (United States) is conducting a population-based trial in Costa Rica. All these phase-III trials aim to demonstrate that vaccines protect against histologically confirmed high-grade CIN associated with the targeted HPV types. Anticipated results of phase-III trials

of the quadrivalent MSD vaccine showed 100% protection against HPV16/18-associated CIN2 and adenocarcinoma in situ in HPV naive women who received the complete vaccine regimen (ATP) (Skjeldestad, 2005).

3.2. Therapeutic HPV vaccines

Development and maintenance of cervical precursors and their progression to invasive cancer requires the continued intra-cellular expression of the viral oncoproteins of E6 and E7 (Steenbergen et al., 2005; Zur Hausen, 2002). Therefore therapeutic vaccines have aimed at stimulating T-cell responses against these viral early oncogenes. Currently, different methods and formats of therapeutic vaccines such as administration of peptide antigens or recombinant proteins, plasmid DNA vaccines, viral vector vaccines, and administration of E7 pulsed dendritic cells, are being evaluated (Stern, 2005). Trials show that these vaccines are safe and variably immunogenic, although there is often no correlation with clinical outcomes (Stern, 2004).

4. Questions about HPV vaccination

4.1. Endpoints

Although cervical cancer is the most important clinically relevant endpoint, it was agreed that surrogate end-points are needed, for two simple reasons: (1) malignancies develop slowly and cancer as an endpoint requires very large and lengthy studies, and (2) state-of-the-art clinical management requires that premalignant lesions are treated immediately, making such an endpoint unfeasible in a clinical trial setting (Pagliusi and Teresa, 2004). On the other hand, evidence of incident HPV infection with a type-specific vaccine is an endpoint that would seem to be an obvious choice for a clinical trial against an infectious disease. However, a high percentage of sexually active women, are at least transiently infected with one or more genital HPV types. Because HPV-induced clinical disease occurs in only a relatively small proportion of infected individuals, estimates of vaccine efficacy cannot be based on protection against infection.

Recently, a WHO expert group reached a consensus and proposed histologically confirmed high-grade CIN or worse disease (including cervical cancer) associated with one of the target vaccine types as an acceptable surrogate endpoint for phase-III vaccination trials (Pagliusi and Teresa, 2004). Type-specific persistence, defined as presence of the same HPV type at two or more consecutive visits separated by 6–12 months, is another interesting outcome measure (Lowy and Frazer, 2003).

Comparing the incidence of cervical and other HPV-associated cancers in vaccinated and non-vaccinated cohorts, by linkage to cancer registries, will provide the ultimate proof of protection against cancer (Lehtinen, 2004, 2005;

Table 1
Study characteristics and main results reported by three randomised placebo-controlled phase-IIb HPV vaccination trials

	Author (year)		
	Koutsky et al. (2002) and Mao et al. (2006)	Harper et al. (2004, 2006)	Villa et al. (2005)
Vaccine	HPV16 L1 VLP, produced in yeast (<i>Saccharomyces cerevisiae</i>)	HPV16 and 18 L1, produced in <i>Spodoptera frugiperda</i> Sf-9 and <i>Trichoplusia ni</i> Hi-5 cell substrate, respectively, via a recombinant baculovirus vector	HPV6, 11,16 and 18 L1 VLP, produced in yeast (<i>Saccharomyces cerevisiae</i>)
Adjuvant	225 mg aluminiumhydroxy-phosphate sulphate	500 mg AIOH and 50 mg 3-deacylated monophosphoryl lipid A	225 mg aluminiumhydroxy-phosphate sulphate
Dosage of VLPs	40 µg	20/20 µg	20/40/40/20 µg
Vaccination schedule	IM injections, 0.5 mL, at 0, 2 and 6 months	IM injections, 0.5 mL, at 0, 1 and 6 months	IM injections, 0.5 mL, at 0, 2 and 6 months
Study size	Randomised: 1194 vaccine/1198 placebo, ATP: 6 M:768 vaccine/765 placebo, ATP: ≥7 M: 755 vaccine/750 placebo	Randomised: 560 vaccine/553 placebo, ATP: 6 M: 540 vaccine/541 placebo, ATP: ≥7 M: 366 vaccine/355 placebo	Randomised: 277 vaccine/275 placebo, ATP: 6 M: 256 vaccine/260 placebo, ATP: ≥7 M: 239 vaccine/242 placebo
Study sites	USA	Brazil, USA, Canada	Brazil, Europe, USA
Inclusion criteria	Women, HPV DNA negative at M0 and M7, HPV16 seronegative at M0	Healthy women, cytologically normal, seronegative for HPV16 and 18. HPV DNA negative for 14 types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68)	Healthy women, accepting contraception, virgins only when looking for contraception
Exclusion criteria	Pregnancy, history of abnormal Pap, ≥6 sex partners	≥6 sex partners, history of abnormal Pap, treatment on cervix, ongoing treatment for external condylomata	Pregnancy, previous abnormal smears, >4 sex partners. Women with a previous HPV infection were NOT excluded
Age range	16–23 years	15–25 years	18–23 years
Duration follow-up	48 months	53 months	36 months
Efficacy: protection against			
Incident infections	At 18 M: 91% (CI: 80–97), at 48 M: not documented	ATP at 53 M: HPV16: 98% (CI: 87–100%), HPV18: 89% (CI: 53–99%), HPV16/18: 95% (CI: 84–99%)	Not documented
Persistent infections	ATP: (infection lasting 4 months or more), at 18 M: 100% (CI: 90–100), At 48 M: 94% (CI: 88–98%)	ATP up to 53 M: (infection lasting 6 months or more), HPV16: 95% (CI: 9–100%), HPV18: 100% (CI: 4–100%), HPV16/18: 96% (CI: 75–100%), protection was 100% for infections lasting 12 months or more.	ATP at 36 M: (infection lasting 4 months or more), HPV6: 100% (68–100), HPV11: not computable* HPV16: 86% (54–97), HPV18: 89% (21–100), HPV6/11/16/18: 89% (CI: 70–97)
Cytological lesions associated with targeted HPV type	Not documented	ATP up to 53 M: HPV16: 97% (CI:82–100), HPV18: 94% (CI: 64–100), HPV16/18: 96% (CI: 84–100)	Not documented
CIN associated with targeted HPV type	ATP at 18 M: 100% (CI: 24–100), at 48 M: 100% (CI: 85–100)	ATP up to 53 M: HPV16: 100% (CI: 42–100), HPV18: not computable*	ATP at 36 M: HPV6/11/16/18: 100% (CI: 23–100)
Seroconversion (at 7 months)	HPV16: 100%	Up to 53 M: HPV16 and 18: 100%	100% for all four types
Increase antibody titre after vaccination (GMTvacc/GMTni), ni = nat infection	At 7 M: (GMTvacc/GMTnat inf), HPV16: ≈58.8, at 48 M: ≈6 (estimated from Fig. 3 in Mao et al. Obstet Gynecol: 2006)	At 7 M: (GMTvacc/GMT placebo), HPV16: 1270, HPV18: 191, at 18 M: (GMTvacc/GMTplacebo), HPV16: 935, HPV18: 137, high titres maintained up to 53 months	At 7 M: (GMTvacc/GMTnat inf), HPV6: 10.6, HPV11: 7.4, HPV16: 105.2, HPV18: 19.1. At 36 M: (GMTvacc/GMTnat inf), HPV6: 1.4, HPV11: 1.0, HPV16: 17.6, HPV18: 2.1

ATP: according-to-protocol analysis; CI: 95% confidence interval; GMT: geometric mean titre of antibodies; ITT: intention-to-treat analysis; VLP: virus like particles. *No cases in vaccinated or placebo group.

Lehtinen et al., 2006a,b). In anticipation of such results, estimations of the impact of HPV vaccination on the burden of cervical cancer incidence and mortality must be based on the observed surrogate endpoints using mathematical modelling (Barnabas et al., 2006; Garnett et al., 2006).

4.2. Duration and consistency of the antibody response to VLPs

The long-term duration of protection against HPV infection, elicited by vaccination is still unknown. Type-specific L1 VLP-antibodies reach maximum titres at month 7, i.e. 1

Table 2
Phase-III vaccination trials, currently being conducted or planned (adapted from Cohen (2005))

Vaccine	Location	Participants	Expected end of the trial
Quadrivalent vaccine containing L1 VLPs of HPV6/11/16/18 produced in yeast (manufactured by MSD)	USA, South-America, Europe	17,800 women, aged 16–26 years	2007
	USA, South-America, Europe, Asia	3800 women aged 24–45 years	2008
	USA, South-America, Europe, Asia, Africa	3700 men aged 16–24 years	2008
Bivalent vaccine containing L1 VLPs of HPV16/18 produced in baculovirus (manufactured by GSK)	USA, South-America, Europe, Asia, Pacific	18,000 women, aged 15–25 years	2010
	Costa Rica (conducted by the National Cancer Institute)	12,000 women, aged 18–25 years	2010

month after administration of the third dose. Titres decline until month 24 and remain rather stable thereafter (Villa et al., 2005, 2006). Nevertheless, at 3 years, antibody titres remain 2–20-fold higher than in placebo controls (Villa et al., 2006).

Complete protection against HPV16 associated CIN lesions was observed over the whole follow-up duration of two phase-IIb trials: 48 months for the monovalent HPV16 vaccine and 53 months for the bivalent HPV16/18 vaccine (Harper et al., 2006; Mao et al., 2006). The use of ASO4 adjuvant (3-*O*-desacyl-4'-monophosphoryl lipid A and aluminium salt) triggers higher virus-neutralizing antibody titres and production of memory B cells compared to VLPs adjuvanted with aluminium salt alone (Giannini et al., 2006). Whether this will result in prolonged enhanced protection against cervical lesions is still unknown.

4.3. Optimal target age range for vaccination

Epidemiological studies indicate that many women become infected within several months of initiation of sexual activity (Koutsky et al., 1992; Winer et al., 2003; Woodman et al., 2001). Therefore, vaccination at an age of 12–14 years, just before initiation of sexual contacts, or at childhood age, perhaps adding a booster in adolescence or early adulthood, seems like an obvious strategy. The protocols of phase-IIb trials have excluded women who were vaccine-type HPV DNA- or sero-positive at enrolment or who became HPV DNA-positive during the administration period. Nevertheless, a reduced protection was observed in a small cohort of non-HPV naïve women who received the HPV16 VLP (Mao et al., 2006). The preliminary analysis of the large phase-III with the quadrivalent vaccine observed that protection against HPV16- or HPV18-associated CIN2+ or AIS was absent among women who were baseline HPV DNA-positive and sero-positive for HPV16 or 18. Protection was strongly reduced (efficacy of 31.2; 95% CI: <0–54.9%) for women who were HPV DNA-positive but sero-negative at the time of vaccination.¹ These data suggest a potential utility of testing for the HPV status before vaccinating women who have already initiated sexual contacts or when vaccinating older women.

¹ See “GARDASIL (Human Papillomavirus [types 6, 11, 16 and 18] Recombinant Vaccine, Vaccines and Related Biological Products Advisory Committee (VRBPAC). Briefing Document”, available at www.fda.gov.

4.4. Immunization of males

Genital tract HPV infection is sexually transmitted. Therefore, immunization of men may help prevent transmission to and infection of women. Modelling studies on herd immunity, i.e. indirect protection of those who remain susceptible, owing to a reduced prevalence of infections in the risk group for disease, have been published (Garnett, 2005; Hughes et al., 2002; Taira et al., 2004). Hughes et al. (2002) determined that vaccinating women alone could reduce the prevalence of infection with the specific HPV type in the vaccinated group by 30%, and that vaccinating both males and females could reduce the prevalence by 44%. Taira et al. (2004) estimated that vaccinating boys would affect cervical cancer incidence only marginally and concluded that it was not cost-effective compared with vaccinating only girls.

Currently, few data are available regarding the immune responses to HPV VLPs in men, although studies are being initiated (Cohen, 2005; Geipert, 2005). Immunisation of men with VLPs is expected to elicit a serum immune response similar to that in women. A major obstacle in testing the efficacy of HPV vaccines in men has been the lack of safe, simple and reliable sampling methods.

4.5. Inclusion of HPV types

Antibody responses elicited by VLP immunization are quite specific for the individual HPV type, with limited cross-neutralisation even for closely related HPV types. Thirteen (or more) different “high risk” types have been identified as causative agents of cervical cancer (Cogliano et al., 2005; Munoz et al., 2003). These considerations raise an important question: “How many different HPV types can be included in prophylactic vaccines, given that each type requires a certain amount of antigen to be included in the preparation?” (Franco and Harper, 2005; Munoz et al., 2004).

Fig. 1 shows the cumulative proportion of the main HPV types present in cervical cancer, estimated for Europe from surveys and population-based case-control studies conducted by IARC (Munoz et al., 2004). It also illustrates the estimated number of cervical cancer cases that can be attributed to the same ranked combination of HPV types. According to the most recent available estimates published in GLOBOCAN-2002, approximately 60,000 new cases of cancer occur yearly in Europe (Ferlay et al., 2004). If all women at risk were vacci-

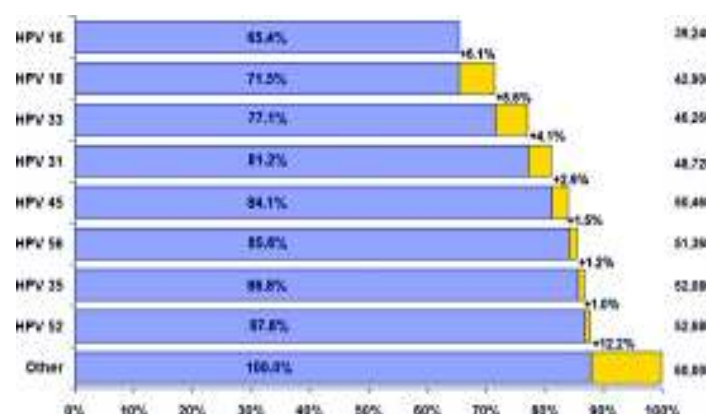


Fig. 1. Cumulative proportion of cervical cancers in Europe that are attributed to a ranked combination of HPV types and the number of cervical cancers occurring each year expected to be caused by these types. In Europe, 60,000 cases of cervical cancer occur yearly. Sixty five percent, or 39,240 cancer cases, are attributed to HPV16. 71.5% (or 6.1% more) can be attributed to HPV16 or HPV18. Almost 88% of cervical cancers are attributed to one of eight HPV types (HPV16, 18, 33, 31, 45, 56, 35 and 52). Adapted from Munoz et al. (2004) and Ferlay et al. (2004).

nated with a 100% effective HPV16 vaccine, 39,000 incident cases of cervical cancer could be avoided. Adding HPV18 type to the vaccine could potentially avoid 43,000 cases per year (71.5%). An octovalent vaccine could potentially reduce the incidence with 88%. This simple extrapolation assumes absence of replacement or cross-protection, which respectively should decrease or increase vaccine efficacy. Replacement means that other HPV types not included in the vaccine cocktail might take over the carcinogenic role of the eliminated types. Follow-up over 5 years of the phase-II trials did not show evidence of such a replacement phenomenon. Moreover, the GSK trial using a bivalent HPV16/18 vaccine with an AS04 adjuvant reported partial cross-protection against infection with HPV types related to HPV16 and 18 was reported (Dubin et al., 2005). Protection was 94.2% (95% CI: 63.3–99.9%) and 54.5% (95% CI: 11.5–77.7%) for incident infection with HPV45 and HPV31, respectively (Harper et al., 2006).

4.6. Combination of screening and HPV vaccination

Current L1 VLP vaccines do not include all oncogenic types. Moreover, since such vaccines are aimed at protecting HPV-naïve individuals, and the effect on women already infected may be low or even absent, screening will continue to be necessary. Setting up vaccination programmes for teenage girls, will have an observable impact on cancer incidence trends only after 2–3 decades.

Nevertheless, vaccination may allow starting screening of vaccinated cohorts at older age, increasing the screening interval and reducing the burden of precursor lesions requiring follow-up and treatment in vaccinated cohorts. Goldie, looking for the most cost-effective strategies, estimated that conventional cytological screening every 5 years starting at 30 years of age could result in 67% reduction in lifetime can-

cer risk. Adding vaccination against HPV16 and 18, assuming 80% efficacy, could yield a reduction of 89% (Goldie et al., 2004).

Health authorities and care providers should understand that screening and vaccination are complementary strategies (Schiller and Davies, 2004). Neglecting screening because vaccination programmes have begun could paradoxically lead to an increase of the cervical cancer burden.

4.7. Vaccination against non-oncogenic HPV

HPV types 6 and 11 jointly cause more than 90% of genital warts (Lacey et al., 2006). Low-grade and even non-progressive high-grade dysplastic lesions of the cervix may be caused by these and other non-oncogenic types as well. Moreover, HPV types 6 and 11 can cause serious disease in rare circumstances. HPV6 and HPV11 are the major cause of recurrent respiratory papillomatosis, a severe disease that may be fatal. So-called giant condylomas or Buschke-Löwenstein tumors of the vulva, penis and anus are also associated with these HPV types (Cogliano et al., 2005). These tumours are regarded as having a low potential for malignancy, but may also be fatal. The vaccine manufactured by Merck contains L1 VLPs of both HPV 6 and HPV 11. Phase-II trials have shown complete protection against external genital lesions but were underpowered to generate statistically significant results (Villa et al., 2005). High clinical and statistically significant protection was confirmed in phase-III trials.²

² Press Release P06-77, 8 June 2006, FDA News, accessible on <http://www.fda.org>.

4.8. Inclusion of HPV proteins in addition to L1 in vaccines

Addition of other proteins to the L1 VLPs requires increased technological challenges and costs. A combination of L1 and L2 appears promising since anti-L2 could protect against heterologous HPV types. The addition of early antigens (E6 or E7 in particular) is also being investigated to determine if a cell-mediated immune response could be elicited along with the antibody response to the L1 VLP component (Greenstone and Nieland, 1998). If so, this would open the way to development of chimeric vaccines with a therapeutic and prophylactic activity (Schiller and Nardelli-Haeffliger, 2006; Stanley, 2003).

5. Licensure of VLP vaccines

On 8 June 2006, the US Food and Drug Administration (FDA) approved Gardasil[®], the quadrivalent vaccine, developed by MSD, containing VLP L1 of HPV types 6, 11, 16 and 18, for use in females 9–26 years of age (see footnote 2). The FDA recognised the indication of protection against cervical cancer, genital warts (condyloma acuminata), cervical adenocarcinoma in situ, cervical intraepithelial neoplasia (grades 2, 3 and also 1), vulvar intraepithelial neoplasia (grades 2 and 3) and, vaginal intraepithelial neoplasia (grades 2 and 3) caused by the vaccine types. The FDA press release stated that the vaccine is effective if administered prior to HPV infection.

The Advisory Committee for Immunization Practices (ACIP) of the CDC, recently recommended routine vaccination of girls of 11–12 years old, but also allowed the administration of the vaccine to girls of 9 or 10 years and girls and young women of 13–26 years of age.³

On 27 July 2006, the Committee for Medicinal Products for Human Use (CHMP) of the European Medicine Agency (EMA) adopted a positive opinion, recommending to grant a marketing authorisation of Gardasil for the prevention of high-grade cervical dysplasia (CIN 2/3), cervical carcinoma, high-grade vulvar dysplastic lesions (VIN 2/3), and external genital warts.⁴ On 20 September 2006, EMA has provided the official authorization for marketing of the vaccine in the European Union, specifying that its use should be in accordance with official recommendations.

An application is also introduced at the EMA for licensure of Cervarix (the bivalent VLP L1 HPV16/18 vaccine manufactured by GSK).

6. Conclusions and recommendations

Results from the phases-IIb and -III trials published thus far indicate that the L1 VLP HPV vaccine is safe and well

tolerated. It offers HPV naïve women a very high level of protection against HPV persistent infection and cervical intra-epithelial lesions associated with the types included in the vaccine.

Currently, only prophylactic HPV vaccines have shown promise. While prophylactic vaccination is likely to provide important future health gains, cervical screening will need to be continued for the whole generation of women that is already infected.

Due to the multiplicity of HPV types and the fact that the coming vaccines are essentially type-specific, the prophylactic vaccines are not likely to eradicate cervical cancer. A reduction in background risk by elimination of the most important HPV types would affect cost-effectiveness and timing/intervals of screening programs, but would not obviate them.

The continuous monitoring of which HPV types are spreading in the population will become necessary, for early monitoring of “fill in” phenomena, inappropriate vaccination strategies or other reasons for vaccination failure.

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References

- Barnabas RV, Laukkanen P, Koskela P, Kontula O, Lehtinen M, Garnett GP. Epidemiology of HPV 16 and cervical cancer in Finland and the potential impact of vaccination: mathematical modelling analyses. *PLoS Med* 2006;3:1–9.
- Bosch FX, Lorincz AT, Munoz N, Meijer CJ, Shah KV. The causal relation between human papillomavirus and cervical cancer. *J Clin Pathol* 2002;55:244–65.
- Carter JJ, Koutsky LA, Wipf GC, Christensen ND, Lee SK, Kuypers J, et al. The natural history of human papillomavirus type 16 capsid antibodies among a cohort of university women. *J Infect Dis* 1996;174:927–36.
- Carter JJ, Koutsky LA, Hughes JP, Lee SK, Kuypers J, Kiviat N, et al. Comparison of human papillomavirus types 16, 18, and 6 capsid antibody responses following incident infection. *J Infect Dis* 2000;181:1911–9.
- Christensen ND, Kreider JW, Kan NC, DiAngelo SL. The open reading frame L2 of cottontail rabbit papillomavirus contains antibody-inducing neutralizing epitopes. *Virology* 1991;181:572–9.

³ <http://www.cdc.gov/nip/vaccine/hpv/>.

⁴ Press Release Doc.Ref. EMA/CHMP/274938/2006, available at <http://www.emea.eu.int/pdfs/human/opinion/Gardasil27493806.pdf>.

- Cogliano V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F. Carcinogenicity of human papillomaviruses. *Lancet Oncol* 2005;6:204.
- Cohen J. Public health. High hopes and dilemmas for a cervical cancer vaccine. *Science* 2005;308:618–21.
- Dillner J. The serological response to papillomaviruses. *Semin Cancer Biol* 1999;9:423–30.
- Dillner J, Kallings I, Brihmer C, Sikstrom B, Koskela P, Lehtinen M, et al. Seropositivity to human papillomavirus types 16, 18 or 33 capsids and to *Chlamydia trachomatis* are markers of sexual behaviour. *J Infect Dis* 1996;173:1394–8.
- Dillner J, Lehtinen M, Bjorge T, Luostarinen T, Youngman L, Jellum E, et al. Prospective seroepidemiologic study of human papillomavirus infection as a risk factor for invasive cervical cancer. *J Natl Cancer Inst* 1997;89:1293–9.
- Dubin G, Colau B, Zahaf T, Quint W, Martin M, Jenkins D. Cross-protection against persistent hpv infection, abnormal cytology and CIN associated with HPV-16 AND 18 related HPV types by a HPV 16/18 L1 virus-like particle vaccine. Vancouver 2005.
- Ferlay J, Bray F, Pisani P, Parkin DM, GLOBOCAN. 2004. Cancer incidence, mortality and prevalence worldwide. IARC CancerBase No. 5, Version 2.0; IARC Press, Lyon, 2004.
- Franco EL, Harper DM. Vaccination against human papillomavirus infection: a new paradigm in cervical cancer control. *Vaccine* 2005;23:2388–94.
- Frazer IH. Prevention of cervical cancer through papillomavirus vaccination. *Nat Rev Immunol* 2004;4:46–55.
- Galloway DA. Papillomavirus vaccines in clinical trials. *Lancet Infect Dis* 2003;3:469–75.
- Garnett GP. Role of herd immunity in determining the effect of vaccines against sexually transmitted disease. *J Infect Dis* 2005;191(Suppl 1):S97–106.
- Garnett GP, Kim JJ, French K, Goldie SJ. Chapter 21: modelling the impact of HPV vaccines on cervical cancer and screening programmes. *Vaccine* 2006;24:178–86.
- Geipert N. Vaccinating men for HPV: new strategy for preventing cervical cancer in women? *J Natl Cancer Inst* 2005;97:630–1.
- Giannini SL, Hanon E, Moris P, Van Mechelen M, Morel S, Dessy F, et al. Enhanced humoral and memory B cellular immunity using HPV16/18 L1 VLP vaccine formulated with the MPL/aluminium salt combination (AS04) compared to aluminium salt only. *Vaccine* 2006;24:5937–49.
- Goldie SJ, Kohli M, Grima D, Weinstein MC, Wright TC, Bosch FX, et al. Projected clinical benefits and cost-effectiveness of a human papillomavirus 16/18 vaccine. *J Natl Cancer Inst* 2004;96:604–15.
- Greenstone HL, Nieland JD. Chimeric papillomavirus virus-like particles elicit antitumor immunity against the E7 oncoprotein in an HPV16 tumor model. *Proc Natl Acad Sci USA* 1998;95:1800–5.
- Harper DM, Franco EL, Wheeler C, Ferris DG, Jenkins D, Schuind A, et al. Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial. *Lancet* 2004;364:1757–65.
- Harper DM, et al. Sustained efficacy up to 4–5 years of bivalent L1 virus-like particle vaccine against human papillomavirus types 16 and 18: follow-up from a randomised control trial. *Lancet* 2006;367:1247–55.
- Hines JF, Ghim SJ, Christensen ND, Kreider JW, Barnes WA, Schlegel R, et al. Role of conformational epitopes expressed by human papillomavirus major capsid proteins in the serologic detection of infection and prophylactic vaccination. *Gynecol Oncol* 1994;55:13–20.
- Ho GY, Studentsov YY, Bierman R, Burk RD. Natural history of human papillomavirus type 16 virus-like particle antibodies in young women. *Cancer Epidemiol Biomarkers Prevent* 2004;13:110–6.
- Hughes JP, Garnett GP, Koutsky L. The theoretical population-level impact of a prophylactic human papilloma virus vaccine. *Epidemiology* 2002;13:631–9.
- IARC Monograph Working Group, et al. IARC monographs on the evaluation of carcinogenic risks to humans, vol 90: human papillomaviruses. Lyon: International Agency for Research in Cancer; 2007, in press.
- Kawana K, Yoshikawa H, Taketani Y, Yoshiike K, Kanda T. Common neutralization epitope in minor capsid protein L2 of human papillomavirus types 16 and 6. *J Virol* 1999;73:6188–90.
- Kirnbauer R, Hubbert NL, Wheeler CM, Becker TM, Lowy DR, Schiller JT. A virus-like particle enzyme-linked immunosorbent assay detects serum antibodies in a majority of women infected with human papillomavirus type 16. *J Natl Cancer Inst* 1994;86:494–9.
- Konya J, Dillner J. Immunity to oncogenic human papillomaviruses. *Adv Cancer Res* 2001;82:205–38.
- Koutsky LA, Holmes KK, Critchlow CW, Stevens CE, Paavonen J, Beckmann AM, et al. A cohort study of the risk of cervical intraepithelial neoplasia grade 2 or 3 in relation to papillomavirus infection. *N Engl J Med* 1992;327:1272–8.
- Koutsky LA, Ault KA, Wheeler CM, Brown DR, Barr E, Alvarez FB, et al. A controlled trial of a human papillomavirus type 16 vaccine. *N Engl J Med* 2002;347:1645–51.
- Lacey CJ, Lowndes CM, Shah KV. Chapter 4: burden and management of non-cancerous HPV-related conditions: HPV-6/11 disease. *Vaccine* 2006;24:35–41.
- Lehtinen M. Vaccination against human papillomaviruses shows great promise. *The Lancet* 2004;364:1731–2.
- Lehtinen M. Preparations for implementing human papillomavirus vaccination should begin. *Eur Surveill* 2005;10:1–2.
- Lehtinen M, Luukkaala T, Wallin KL, Paavonen J, Thoresen S, Dillner J, et al. Human papillomavirus infection, risk for subsequent development of cervical neoplasia and associated population attributable fraction. *J Clin Virol* 2001;22:117–24.
- Lehtinen M, Apter D, Dubin G, Kosunen E, Isaksson R, Korpiavaara EL, et al. Enrolment of 22,000 adolescent women to cancer registry follow-up for long-term human papillomavirus vaccine efficacy: guarding against guessing. *Int J STD AIDS* 2006a;17:517–21.
- Lehtinen M, Idanpaan-Heikkilä I, Lunnas T, Palmroth J, Barr E, Cacciatore R, et al. Population-based enrolment of adolescents in a long-term follow-up trial of human papillomavirus vaccine efficacy. *Int J STD AIDS* 2006b;17:237–46.
- Lowy DR, Frazer IH. Chapter 16: prophylactic human papillomavirus vaccines. *J Natl Cancer Inst Monogr* 2003:111–6.
- Man S. Human cellular immune responses against human papillomaviruses in cervical neoplasia. *Expert Rev Mol Med* 1998;1998:1–19.
- Mao C, Koutsky LA, Ault KA, Wheeler CM, Brown DR, Wiley DJ, et al. Efficacy of human papillomavirus-16 vaccine to prevent cervical intraepithelial neoplasia: a randomized controlled trial. *Obstet Gynecol* 2006;107:18–27.
- Munoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, Shah KV, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med* 2003;348:518–27.
- Munoz N, Bosch FX, Castellsague X, Diaz M, de Sanjose S, Hammouda D, et al. Against which human papillomavirus types shall we vaccinate and screen? The international perspective. *Int J Cancer* 2004;111:278–85.
- Munoz N, Castellsague X, de Gonzalez AB, Gissmann L. Chapter 1: HPV in the etiology of human cancer. *Vaccine* 2006;24:1–10.
- Niedergang F, Didierlaurent A, Kraehenbuhl JP, Sirard JC. Dendritic cells: the host Achilles's heel for mucosal pathogens? *Trends Microbiol* 2004;12:79–88.
- Nieland JD, Da Silva DM. Chimeric papillomavirus virus-like particles induce a murine self-antigen-specific protective and therapeutic anti-tumor immune response. *J Cell Biochem* 1999;73:145–52.
- Orth G, Favre M. Human papillomaviruses. Biochemical and biologic properties. *Clin Dermatol* 1985;3:27–42.
- Pagliusi SR, Teresa AM. Efficacy and other milestones for human papillomavirus vaccine introduction. *Vaccine* 2004;23:569–78.
- Parkin DM, Bray F. Chapter 2: the burden of HPV-related cancers. *Vaccine* 2006;24(Suppl 3):S11–25.
- Roden RB, Hubbert NL, Kirnbauer R, Christensen ND, Lowy DR, Schiller JT. Assessment of the serological relatedness of genital human papillomaviruses by hemagglutination inhibition. *J Virol* 1996;70:3298–301.

- Roden RB, Yutzy WH, Fallon R, Inglis S, Lowy DR, Schiller JT. Minor capsid protein of human genital papillomaviruses contains subdominant, cross-neutralizing epitopes. *Virology* 2000;270:254–7.
- Schiller JT, Davies P. Delivering on the promise: HPV vaccines and cervical cancer. *Nat Rev Microbiol* 2004;2:343–7.
- Schiller JT, Nardelli-Haeffliger D. Chapter 17: second generation HPV vaccines to prevent cervical cancer. *Vaccine* 2006;24:147–53.
- Schneider A, Gissmann L. Cervical cancer. The potential role of human papillomavirus (HPV)-specific vaccines in prevention and treatment. *Am J Cancer* 2003;2:1–253.
- Shah KV, Viscidi RP, Alberg AJ, Helzlsouer KJ, Comstock GW. Antibodies to human papillomavirus 16 and subsequent in situ or invasive cancer of the cervix. *Cancer Epidemiol Biomarkers Prevent* 1997;6:233–7.
- Skjeldestad FE. Prophylactic quadrivalent human papillomavirus (HPV) (types 6, 11, 16, 18) L1 virus-like particle (VLP) vaccine (Gardasil™) reduces cervical intraepithelial neoplasia (CIN) 2/3 risk, 43rd Annual Meeting of IDSA, San Francisco; 2005.
- Stanley MA. Progress in prophylactic and therapeutic vaccines for human papillomavirus infection. *Exp Rev Vaccines* 2003;2:381–9.
- Steenbergen RD, de Wilde J, Wilting SM, Brink AA, Snijders PJ, Meijer CJ. HPV-mediated transformation of the anogenital tract. *J Clin Virol* 2005;32(Suppl 1):S25–33.
- Stern PL. Recent developments in human papillomavirus vaccines. *Exp Opin Invest Drugs* 2004;13:959–71.
- Stern PL. Immune control of human papillomavirus (HPV) associated anogenital disease and potential for vaccination. *J Clin Virol* 2005;32(Suppl 1):S72–81.
- Taira AV, Neukermans CP, Sanders GD. Evaluating human papillomavirus vaccination programs. *Emerg Infect Dis* 2004;10:1915–23.
- Tjalma WA, Arbyn M, Paavonen J, Van Waes TR, Bogers JJ. Prophylactic human papillomavirus vaccines: the beginning of the end of cervical cancer. *Int J Gynecol Cancer* 2004;14:751–61.
- Villa LL, Costa RLR, Petta CA, Andrade RP, et al. Prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in young women: a randomised double-blind placebo-controlled multicentre phase II efficacy trial. *Lancet Oncol* 2005;6:271–8.
- Villa LL, Ault KA, Giuliano AR, Costa RL, Petta CA, Andrade RP, et al. Immunologic responses following administration of a vaccine targeting human papillomavirus types 6, 11, 16, and 18. *Vaccine* 2006;24:5571–83.
- Viscidi RP, Schiffman MA, Hildesheim A, Herrero R, Castle PE, Bratti MC, et al. Seroreactivity to human papillomavirus (HPV) types 16, 18, or 31 and risk of subsequent HPV infection: results from a population-based study in Costa Rica. *Cancer Epidemiol Biomarkers Prevent* 2004;13:324–7.
- White WI, Wilson SD, Palmer-Hill FJ, Woods RM, Ghim SJ, Hewitt LA, et al. Characterization of a major neutralizing epitope on human papillomavirus type 16 L1. *J Virol* 1999;73:4882–9.
- Winer RL, Lee SK, Hughes JP, Adam DE, Kiviat NB, Koutsky LA. Genital human papillomavirus infection: Incidence and risk factors in a cohort of female university students. *J Epidemiol* 2003;157:218–26.
- Woodman CB, Collins S, Winter H, Bailey A, Ellis J, Prior P, et al. Natural history of cervical human papillomavirus infection in young women: a longitudinal cohort study. *Lancet* 2001;357:1831–6.
- Zhou J, Sun XJ, Stenzel DJ, Frazer IH. Expression of vaccinia recombinant HPV16 L1 and L2 ORF proteins in epithelial cells is sufficient for assembly of HPV virion-like particles. *Virology* 1991;185:251–7.
- Zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. *Nat Rev Cancer* 2002;2:342–50.

Addendum

Important new evidence has become available since the preparation of the preceding review on HPV vaccination (Arbyn & Dillner, 2007). Furthermore, some statements in the review, if taken out of context, might be understood as general recommendations to implement HPV vaccination or testing policies or practices. We would like to point out that testing of unsymptomatic women for prevention of cervical cancer is recommended in the current, second edition of the European Guidelines for Quality Assurance in Cervical Cancer Screening to take place only within organised screening programme settings. Moreover, primary HPV testing for cervical cancer screening is currently only recommended in pilot studies (see chapters 2 and 3).

As a matter of editorial policy, the current Guideline edition does not provide recommendations on implementation of HPV vaccination. Development of comprehensive European guidelines on prevention of cervical cancer which appropriately integrate screening and vaccination strategies is a key aim of the next phase of project activities supported by the EU Public Health Programme.

1.1 Efficacy against HPV16/18-specific CIN2+ or AIS in women with current or prior HPV infection

In May 2007, the 3-year follow-up results of the FUTURE I and FUTURE II phase-III trials were published (FUTURE II Study Group 2007; Garland *et al.*, 2007). A major aim of these studies was to evaluate protection of prophylactic vaccination with Gardasil® against vaccine-type-related CIN2+, AIS, cervical cancers (FUTURE I and FUTURE II) and external anogenital lesions (FUTURE I). Prior exposure to vaccine HPV types was assessed by measuring the presence of type-specific antibodies at day 1 and by testing for HPV DNA of vaccine types at day 1 and at 1 month after application of the last vaccine dose.

Excellent protection was observed at month 36 against HPV16/18-related CIN2+ lesions in HPV-naïve women who received all three vaccine doses according to the prescribed protocol: 100% (95% CI: 86-100%) for CIN2, 97% (95% CI: 79-100%) for CIN3, and 100% (95% CI: <0-100%) for AIS in the FUTURE II trial, and 100% for any HPV 16/18-related CIN2+ or AIS in the FUTURE I trial (FUTURE II Study Group 2007; Garland *et al.*, 2007). No observed cases of invasive cervical cancer were reported in the studies.

Among all vaccinated trial participants, protection against HPV16/18-related CIN2+ or AIS was 44% (95% CI: 26-58%). The protection against CIN2+/AIS related to HPV16/18 infection stratified by initial HPV status in the intention-to-treat (ITT) population of the FUTURE II trial is shown in Table 1. The ITT population included all randomised women in the intervention group who received at least one vaccine dose, i.e., also women who were not HPV16 or HPV18 naïve, due to infection prior to, at the onset of, or during the inoculation phase. Women who were HPV16 or HPV18 DNA positive were not protected against CIN2+/AIS related to these types. However, women who were HPV DNA negative for the respective vaccine types showed nearly complete protection. Serological status did not modify the outcomes (FUTURE II 2007).

Table 1. Efficacy at 36 months of the Gardasil® vaccine against CIN2+ or AIS related to HPV16/18 infection, according to initial HPV DNA or serology status (FUTURE II trial, intention-to-treat analysis)-(adapted from: Future II Working Group 2007 and Supplement to same article)

Initial HPV status	Gardasil ®			Placebo			Efficacy	
	N	Cases	Rate /100 PY	N	Cases	Rate /100 PY	%	95% CI
Total	6,087	83	<0.1	6080	148	0.8	44.0%	26-58%
PCR-, sero-	5305	1	<0.1	5260	42	0.3	97.6%	88-100%
PCR-, sero+	498	0	0.00	524	4	0.3	100.0%	<0-100%
PCR+, sero-	423	33	2.9	402	35	3.2	10.6%	<0-46%
PCR+, sero+	298	47	6.1	332	52	6.2	1.2%	<0-35%

Effect modification by type-specific serology was suggested from results pooled from several vaccination trials at 24 months follow-up, which were released by FDA on the occasion of the licensure of Gardasil in the USA (see Arbyn & Dillner, 2007).

The protection against HPV16/18-related CIN2+ or AIS observed at 36 months in the ITT data pooled from two phase II trials and the two phase III FUTURE trials was exactly the same as in the FUTURE II trial: 44% (95% CI: 31-55%) (Ault 2007). The cumulative incidence of vaccine-type-related disease in vaccinated women in the ITT population stabilised after 24 months of time, but continued to rise in non-vaccinated women (FUTURE II Study Group 2007). This observation indicates that vaccine protection would increase with longer follow-up time

Ferris (2006) reported that being HPV DNA positive or serology positive for one vaccine type at vaccination did not alter the excellent protection against disease associated with the other vaccine HPV types with which a woman was not infected (Ferris, 2006).

In the Costa Rican phase III trial with the bivalent Cervarix® vaccine, there was no difference in the 6-to-12-month clearance rate of prevalent HPV 16/18 among women receiving the vaccine (35.5%) versus control (31.5%) (Hildesheim & Herrero, 2006; Ames & Gravitt, 2007). This data suggest that vaccination does not help to clear current infection.

1.2 Efficacy against CIN2+/AIS irrespective of HPV type

Combined results have been reported on the protection against lesions associated with any HPV type in the intention-to-treat population pooled from the FUTURE trials and two phase II trials (Ault 2007). The pooled vaccine efficacy was 18% (95% CI: 7-29 %) for CIN2+/AIS, 21% for CIN2 (95% CI: 7-33%), 17% (95% CI: -0.1-31%) for CIN3 and 57% (95% CI: -19-87%) for AIS. In the vaccinated women, lesions occurred because of prevalent HPV16 or HPV18 infection (present at or during inoculation) or because of prevalent and incident infection with other types.

The FUTURE II study group report also included some information on the effect of the vaccine on any high-grade cervical lesions (any CIN2+ or AIS, irrespective of HPV type) in women tested negative for both HPV16 and HPV18 at enrolment (4693 in the vaccination group and 4703 in the placebo group): high-grade cervical disease developed in 95 subjects in the vaccination group and in 130 in the placebo group; a reduction of 27% (95% CI, 4 to 44%) was reported for the vaccination group. In this population, occurrence of CIN2+ in the vaccinated subjects was almost exclusively due to non-HPV16/18 types. This effect represents reduction of the burden of cervical disease in a completely HPV16/18-naïve population. The observed protection of 27% is lower than that suggested by meta-analyses of the association between cervical cancer precursors and HPV16 or HPV18

types¹, and could suggest occurrence of type replacement (Clifford *et al.*, 2003; Sawaya & Smith-McCune, 2007).

The number of **CIN2+/AIS lesions associated with non-HPV16/18 types** can be computed from the number of CIN2+/AIS lesions associated with any HPV type and the number associated with HPV16 or HPV18. Lower rates of non-HPV16/18-related lesions in the vaccinated group compared to placebo group could be interpreted as an indication for cross-protection, whereas higher rates would suggest occurrence of vaccine-induced type replacement. In the ITT population, there were 252/10,291 (=2.4%) non-HPV16/18 cases of CIN2+/AIS in vaccinated women, versus 228/10,292 (=2.2%) cases in the placebo women². This corresponds to a vaccine efficacy of -11% (95% CI: -32 to 7%). Stratified by histological grade, the vaccine efficacy for protection against non-HPV16/18-related disease was -4% (95% CI: -27 to 15%) for CIN2, -19% (95% CI: -56 to 9%) for CIN3 and 100% (95% CI: <0 to 100%) for AIS. These data do not provide statistical evidence of vaccine-induced CIN2+ caused by type replacement. However, more detailed, type-specific analyses, uniformly defined through trials and stratified by initial HPV infection status, are required to disentangle the type-specific events that determine the vaccine impact on the burden of cervical cancer precursors. In populations in which HPV vaccination will be introduced, potential occurrence of type replacement, escape viral mutants and cross-protection should be evaluated by careful surveillance (Dillner *et al.*, 2007; Arbyn & Dillner, 2007). Furthermore, under routine conditions most women in Europe would not attend cervical screening examinations in the comparatively short examination intervals of 6 or 12 months followed in the previously reported phase III trials. Phase IV studies will therefore also be required to fully evaluate the impact of vaccination under routine conditions.

1.3 Conclusions

Published Phase II trial results have provided evidence that both the quadrivalent and bivalent vaccine induce type-specific antibodies lasting for at least 5 years and protect against persistent infection and cervical lesions associated with the vaccine types.

The recently published phase III studies demonstrate that administering the quadrivalent vaccine to women not infected with vaccine HPV types at the outset of or during the inoculation period, yields excellent protection against CIN2+/AIS and ano-genital lesions, associated with these HPV types, whereas women infected with vaccine HPV types are not protected.

Efficacy was much lower against CIN2+ lesions of any HPV type, even in women not previously or currently exposed to HPV16 or 18.

Outcomes of the currently ongoing phase III trials with the bivalent vaccine are not yet available, but similar excellent efficacy against HPV16/18-related cervical lesions has been shown in phase II trials.

Current evidence does not justify modification of the current guideline recommendations on the age groups and interval for cervical cancer screening in women who have been vaccinated for HPV.

¹ From meta-analyses pooling data from all continents on the association between HPV16/18 and different degrees of cervical lesions, it was estimated that elimination of HPV16/18 would decrease the burden of high-grade cervical intraepithelial lesions by 41-57% (Clifford *et al.*, 2006).

² In vaccinated women included in the ITT populations of the phase III (FUTURE) trials or phase II trials, 394 cases of CIN2+/AIS related to any type and 142 cases of CIN2+/AIS related to HPV16/18 were counted. This means that 394-142=252 were related to non16/18 types. In the placebo group, the corresponding Fig.s were 483-255=228 (Ault, 2007).

In populations in which HPV vaccination will be introduced, potential occurrence of type replacement, escape viral mutants and cross-protection should be evaluated by careful surveillance (Dillner *et al.*, 2007; Arbyn & Dillner, 2007).

1.4 References

Ames A. & Gravitt P. (2007). Human papillomavirus vaccine update. *Curr. Infect. Dis. Rep.* **9**, 151-158.

Arbyn M. & Dillner J. (2007). Review of current knowledge on HPV vaccination: An appendix to the European Guidelines for Quality Assurance in Cervical Cancer Screening. *J. Clin. Virol.* **38**, 189-197.

Ault K.A. (2007). Effect of prophylactic human papillomavirus L1 virus-like-particle vaccine on risk of cervical intraepithelial neoplasia grade 2, grade 3, and adenocarcinoma in situ: a combined analysis of four randomised clinical trials. *Lancet* **369**, 1861-1868.

Clifford G., Franceschi S., Diaz M., Muñoz N., & Villa L.L. (2006). Chapter 3: HPV type-distribution in women with and without cervical neoplastic diseases. *Vaccine* **24**, 26-34.

Clifford G.M., Smith J.S., Aguado T., & Franceschi S. (2003). Comparison of HPV type distribution in high-grade cervical lesions and cervical cancer: a meta-analysis. *Brit J Cancer* **89**, 101-105.

Dillner J., Arbyn M., & Dillner L. (2007). Translational mini-review series on vaccines: Monitoring of human papillomavirus vaccination. *Clin. Exp. Immunol.* **148**, 199-207.

Ferris D. Efficacy of a quadrivalent HPV (types 6/11/16/18) L1 virus-like particle (VLP) vaccine in women with virologic evidence of HPV infection: a combined analysis [Abstract S11-2]. EUROGIN. 2006. Paris. European Research Organization on Genital Infection and Neoplasia. 26-4-2006. Ref Type: Conference Proceeding

FUTURE II Study Group (2007). Quadrivalent vaccine against human papillomavirus to prevent high-grade cervical lesions. *N. Engl. J Med.* **356**, 1915-1927.

Garland S.M., Hernandez-Avila M., Wheeler C.M., Perez G., Harper D.M., Leodolter S., Tang G.W., Ferris D.G., Steben M., Bryan J., Taddeo F.J., Railkar R., Esser M.T., Sings H.L., Nelson M., Boslego J., Sattler C., Barr E., & Koutsky L.A. (2007). Quadrivalent vaccine against human papillomavirus to prevent anogenital diseases. *N. Engl. J Med.* **356**, 1928-1943.

Hildesheim A. & Herrero R. Effect of a HPV 16/18 vaccine on resolution of infections in women with pre-existing HPV. International Papillomavirus Society. 2006. Prague. 23rd International Papillomavirus Conference & Clinical Workshop. 1-7 September 2006.

Markowitz L.E., Dunne E.F., Saraiya M., Lawson H.W., Chesson H., & Unger E.R. (2007). Quadrivalent Human Papillomavirus Vaccine: Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm. Rep.* **56**, 1-24.

Saslow D., Castle P.E., Cox J.T., Davey D.D., Einstein M.H., Ferris D.G., Goldie S.J., Harper D.M., Kinney W., Moscicki A.B., Noller K.L., Wheeler C.M., Ades T., Andrews K.S., Doroshenk M.K., Kahn K.G., Schmidt C., Shafey O., Smith R.A., Partridge E.E., & Garcia F. (2007). American Cancer Society Guideline for human papillomavirus (HPV) vaccine use to prevent cervical cancer and its precursors. *CA Cancer J. Clin.* **57**, 7-28.

HPV VACCINATION

Sawaya G.F. & Smith-McCune K. (2007). HPV vaccination--more answers, more questions. *N. Engl. J Med.* **356**, 1991-1993.

Wheeler C.M. (2007). Advances in primary and secondary interventions for cervical cancer: human papillomavirus prophylactic vaccines and testing. *Nat. Clin. Pract. Oncol.* **4**, 224-235.

Glossary of terms

Background incidence rate	The cervical cancer incidence rate expected in the absence of screening. It is not directly observable, but is estimated as the incidence in the target population before screening started (and adjusted for a trend), or as the simultaneous incidence in a non-screened referent population.
Call-recall system	Invitation system by which women identified as eligible to attend screening are personally invited (generally by letter) and non-attenders are reinvited. Note that some screening programmes may not invite all eligible women. Instead only those women may be invited who have not attended screening within a certain period of time. Recall may also refer to re-invitation to attend subsequent rounds of screening. It also refers to a request or a reminder for follow-up of suspicious smears or for a new smear when the previous was unsatisfactory.
Cause of death register	Records of information on all deaths occurring in a defined population.
Cervical cancer incidence rate	The rate at which new cases of cervical cancer occur in a population. The numerator is the number of new cases of cervical cancer diagnosed in a defined period. The denominator is the sum of women-years, contributed by all members of the population at risk of a new diagnosis of cervical cancer during the defined period. A good approximation of the population at risk is: (average population) * (length of the period). For the yearly incidence rate, an acceptable approximation of the population at risk is the mid-year population, i.e.: (the population at risk at the beginning of the year + the population at the end of the year) / 2. Women hysterectomised for a non-neoplastic indication can be subtracted from the population at risk. However, information on hysterectomy often is not available at the level of the cancer registry or the office for vital statistics.
Cervical cancer mortality rate	The rate at which deaths due to cervical cancer occur in a population. The numerator is the number of cervical cancer deaths that occur in a defined time period. For the denominator: see cervical cancer incidence rate.
Cancer register	Recording of information on all new cases and possibly of deaths from cancer occurring in a defined population.
Cold coagulation	Ablative procedure to treat CIN, uses a probe similar to a cryo-cautery probe, but destroys the tissue by heating it to 100°C.
Cold knife conisation	Excision of a cone-shaped area of the cervix, to include part of the endo-cervical canal, using a knife.

Compliance rate	Proportion of invited women who participate in the programme (=participation rate).
Coverage by invitation	Proportion of the target population to which invitations are sent within the screening interval defined by programme policy.
Coverage by smear tests	Proportion of the target population screened at least once within the screening interval defined by programme policy. Also referred to as coverage by examination.
Cryocautery	Synonymous term for cryotherapy.
Cryotherapy	Ablative procedure to treat CIN, using a probe which is applied directly to the cervix and which freezes the tissue to a depth of 3-4 mm.
Delay time	The time between occurrence of a detectable cervical lesion (destined to become a cancer) and the actual detection of that lesion or cancer by screening. Not directly observable. Delay time= DPCP – lead time.
Detectable preclinical phase (DPCP)	The time between occurrence of a detectable cervical lesion (destined to become a cancer) and detection of a clinically manifest cancer. Sojourn time is a synonymous term. DPCP = delay time + lead time.
Detection rate	The number of histologically confirmed lesions detected at screening per (100 or 1000) women screened.
Diathermocoagulation	Ablative procedure which consists in using heat to destroy cervical epithelium only to a depth of 2-3 mm. The depth of destruction is too superficial for it to be recommended for the treatment of CIN.
Efficacy	The reduction in cervical cancer mortality and/or incidence in randomised trials, i.e., under ideal conditions. Sometimes used also to describe the effect among those screened. In the latter definition, an adjustment for the selection bias among attendants is required.
Effectiveness	The reduction in cervical cancer mortality and/or incidence achieved under real conditions by screening the target population.
Efficiency	Cost-effectiveness.
Eligible population	The population of women eligible to attend screening. The eligible population is equal to or less than the target population, depending on whether or not eligibility criteria are defined by programme policy. In some screening programmes, specifically defined groups of women in the target population are not eligible to attend screening, e.g., due to hysterectomy for a non-neoplastic indication, previous or current cervical cancer, or personal request not to be invited.

Episode sensitivity	The proportion of persons with disease detected by screening among those persons with disease who attend screening. Not directly observable, several methods may be used for estimation. See also screening episode.
Fail-safe system	System aimed to maximise follow-up compliance, by sending reminders to a woman or her smear taker when recommended follow-up (diagnosis or treatment) does not occur in due time.
Further assessment	Additional diagnostic steps (repeat smears, HPV testing, colposcopy, histology) performed to clarify the nature of an abnormality detected by the screening test, either at the time of screening or on recall.
Initial screening	First screening examination of individual women within the screening programme, regardless of the organisational round in which women are screened.
Interval cancer (test)	A primary cervical cancer diagnosed in a woman after a negative screening test, but before the next invitation to screening is due, or within a period equal to a screening interval for a woman who has reached the upper age limit to attend screening.
Interval cancer (episode)	A primary cervical cancer diagnosed in a woman after a negative screening episode (i.e. after a negative screening test or a negative follow-up after a positive screening test) either before the next invitation to screening is due, or within a period equal to a screening interval for a woman who has reached the upper age limit for screening.
Interval cancer rate	Interval cancers divided by person-years accumulated by women with a negative screening test (or a negative screening episode) during the period before the next invitation to screening is due, or within a period equal to a screening interval for a women who have reached the upper age limit for screening
Invasive cervical cancer detection rate	The number of histologically confirmed invasive cancers of the cervix uteri detected at screening per (1000) women screened.
Laser excision conisation	Removal of a cone biopsy using a CO ₂ laser in cutting mode.
Laser vaporisation	Ablative procedure to treat CIN. A CO ₂ laser is used at a high-power setting, under colposcopic control. The laser beam is aimed directly at the tissue to be destroyed.
Lead time	Period between the detection of a lesion by screening and the time point that it should have progressed, in the absence of screening, to a clinically recognised cancer. Not directly observable (see also delay time and sojourn time). Lead time = DPCP - delay time.
LEEP	Loop electro-surgical excision procedure. Synonymous term for LLETZ, used in North-America.
Length bias	The bias towards detection by screening of cancers with longer sojourn times, and therefore with a better prognosis.

Lesion	Screening programme policy must define what a screen-detected lesion should be. A typical definition would be a histologically confirmed CIN1+ case.
LLETZ	Large loop excision of transformation zone using a diathermy wire loop.
NETZ	Needle excision of transformation zone: a technique to treat CIN that uses a straight diathermy needle.
Organised screening	Organised screening programmes require a specific screening policy (specifying targeted population groups and the screening test, intervals and other procedures) and a team at the national or regional level responsible for implementing the policy, i.e., for organizing the delivery of the screening services, maintaining requisite quality, and reporting on performance and results. In addition, a quality-assurance structure is required and a means of ascertaining the population burden of the disease should be available. Population-based screening programmes generally require a high degree of organisation.
Opportunistic screening	Screening performed outside of an organised programme, i.e., in a setting providing health care for patients, and without identification and personal invitation of each woman in the eligible target population. The initiative to perform a screening examination is taken on an individual basis by the woman or the health care provider In contrast to organised screening, the other steps in the screening process and the professional and organisational management of the screening service are generally poorly defined by programme policy, rules and regulations. Quality assurance, monitoring and evaluation are underdeveloped due, among other things, to the lack of a population-based approach to implementation.
Over-diagnosis with screening	Detection of cervical cancers or pre-cancerous lesions in screening that might never have progressed to a clinically recognisable cancer during a woman's lifetime.
Participation rate	Number of women who have a screening test, as a proportion of all women invited to screening (=compliance rate).
Population-based screening	Population-based screening means that the women in the eligible target population in the area served by a screening programme are individually identified and personally invited to attend screening.
Positive predictive value	Proportion of all positive tests (using a defined positivity criterion) at screening that lead to a diagnosis of cancer or of histologically confirmed cervical intra-epithelial neoplasia of a defined degree.
Programme sensitivity	The proportion of women with disease detected by screening among those women with disease invited to attend the screening programme.

Radical diathermy	Ablative procedure to treat CIN, which aims to destroy cervical tissue to a depth of approximately 1 cm, using a straight electro-diathermy needle.
Recall	Request that a woman return to the screening unit, as a consequence of the screening examination, for a repeat test because of technical inadequacy of the screening test (technical recall); or for clarification of an abnormality detected at screening, by performing an additional procedure.
Refined mortality	Mortality rate among women, excluding those in whom cervical cancer was diagnosed before screening began (i.e., incidence-based mortality). Usually the disease-specific mortality is used.
Relative survival rate	Observed survival in the patient group, divided by the expected survival of a comparable group in the general population. Expected survival is estimated from population life tables stratified usually by age, gender and calendar time. Deaths from any cause contribute. A relative survival rate (RSR) of 100% indicates that mortality in the patient group was equivalent to that of the general population during the specified interval. RSR below 100% indicates lower survival than expected, or excess mortality due to the disease.
Screening episode	For any given woman attending screening, the series of events in the screening process beginning with the screening test and including any further assessment based on the test.
Screening interval	Fixed interval between routine screenings defined by the policy of each programme. In most of the cytological screening programmes, the screening interval is 3 or 5 years.
Screening policy	Policy of the screening programme that defines the targeted age group, the geographical area, the screening interval and the screening method.
Screening test	Test applied to all women participating in the programme. Cervical cytology is currently the recommended screening test, but, in the future, it could be another test, e.g., the HPV test.
Sojourn time	Length (duration) of the detectable pre-clinical phase.
Subsequent screening	All screening examinations of individual women within the screening programme following an initial screening examination, regardless of the organisational screening round in which women are screened. There are two types of subsequent screening examinations: • subsequent screening at the regular screening interval, i.e., in accordance with the routine interval defined by the screening policy (SUBS-R). • subsequent screening at irregular intervals, i.e. screening examinations of women who miss an invitation to routine screening and return in a subsequent organisational screening round (SUBS-IRR).

SWETZ	Straight-wire excision of the transformation zone. Synonymous term for NETZ.
Target population	All women residing in the catchment area of a screening programme who are in the age group to whom screening is offered, as defined by screening policy.
Test sensitivity	The proportion of women with positive screening test results among the diseased women attending screening.
Test specificity	The proportion of women with negative screening test results among the women attending screening who are free of disease. The test specificity can neither be assessed in a screening programme nor in a randomised trial comparing alternative screening tests. However, cross-sectional test specificity for one screening episode can be approximated using the following formula: (number of test negatives) / (number of screened women – number of cases confirmed as true positive).
Verification bias	Bias in the estimation of the diagnostic validity of a test when different fractions of screen positives and negatives are verified with the gold standard. Verification bias results in overestimation of sensitivity and underestimation of specificity. Methods exist to adjust for verification bias.

Remarks:

- All measures of test positivity require the definition of a positivity criterion: for instance, ASC-US or worse disease (ASC-US+) or LSIL+.
- All measures of accuracy (sensitivity, specificity, predictive value) require the definition of a test positivity criterion and the definition of disease verified by an acceptable gold standard, for instance, presence of histologically confirmed CIN3 or cancer (CIN3+).
- Moreover, in the definition of the accuracy of a test used in screening, the time perspective must be defined: cross-sectional (current presence), or longitudinal (over x years, with specification of x)

List of Abbreviations

AGC	atypical glandular cells
AGUS	atypical glandular cells of undetermined significance
AIS	adenocarcinoma in situ
ALTS	ASCUS/LSIL triage study
ASC-H	atypical squamous cells, high-grade squamous lesion cannot be excluded
ASCUS	atypical squamous cells of undetermined significance (according to the terminology of the Bethesda System, version 1991)
ASC-US	atypical squamous cells of undetermined significance (according to the terminology of the Bethesda System, version 2001)
ASR	age standardised rate
ATP	according to protocol
bp	base pairs
BSCC	British Society for Clinical Cytology
CCPRB	Cancer Control using Population-based Registries and Bio-banking
CGIN	cervical glandular intra-epithelial neoplasia
CHMP	Committee for Medicinal Products for Human Use
CI	95 % confidence interval
CIN	cervical intra-epithelial neoplasia
CIS	carcinoma in situ
CP	conventional Pap smear
CTZ	congenital transformation zone
DG SANCO	Health and Consumer Protection Directorate General of the European Commission
DNA	desoxyribo-nucleic acid
DR	detection rate
EC	endocervical cells
ECC	endocervical curettage
ECN	European Cancer Network
EFCS	European Federation of Cytology Societies
EMA	European Medicines Agency

ENCCS	European Network for Cervical Cancer Screening
EU	European Union
EUNICE	European Network for Indicators on Cancer
FDA	Food and Drug Administration (USA)
FIGO	Fédération internationale d'Obstétrique et de Gynécologie
FN	false negative
FP	false positive
GMT	geometric mean titre
GP	general practitioner
H & E	haematoxylin and eosin
HC	Hybrid Capture
HPV	human Papillomavirus
hrHPV	high-risk HPV type
HSIL	high-grade squamous intra-epithelial lesion
IARC	International Agency for Research on Cancer
ICD	international classification of diseases
IFCPC	International Federation for Cervical Pathology and Colposcopy
ISO	International Organisation for Standardisation
ITT	intention to treat
IUD	intra-uterine device
LBC	liquid-based cytology
LEEP	loop electrosurgical excision procedure
LLETZ	large loop excision of the transformation zone
lrHPV	low risk HPV type
LSIL	low-grade squamous intraepithelial lesion
NETZ	needle excision of the transformation zone
NHS	National Health Services (United Kingdom)
NHSCSP	National Health Services Cervical Screening Programme
NISH	non-isotopic in situ hybridisation
NPV	negative predictive value

OR	odds ratio
PCR	polymerase chain reaction
PPV	positive predictive value
QA	quality assurance
QC	quality control
QUATE Committee	Committee for Quality Assurance Training and Education
RCT	randomised clinical trial
RLB-analysis	Reversed line blot analysis
RLU	relative light units
RNA	ribo-nucleic acid
RP	rapid previewing or rapid prescreening
RR	relative risk; rapid reviewing
RSR	relative survival rate
RT-PCR	real time PCR
SBLB	satisfactory but limited by
SCJ	squamo-columnar junction
SFP	short PCR fragment
SUBS-IRR	subsequent screening at irregular intervals
SUBS-R	subsequent screening at the regular screening interval
SWETZ	straight wire excision of the transformation zone
TBS	The Bethesda System
TN	true negative
TP	true positive
TZ	transformation zone
VAIN	vaginal intra-epithelial neoplasia
VIN	vulvar intra-epithelial neoplasia
VLP	virus-like particles
WHO	World Health Organisation

