An investigation of specific contributing factors affecting quality assurance in the diagnosis of conventional Cervical smears

By

Suzette Mirietta Jordaan
8809372

Submitted in complete fulfillment of the requirements for the Magister Technologiae:
Biomedical technology at the
Nelson Mandela Metropolitan University

January 2005

Promoter: Dr. Nanette Smith
Co-promoter: Dr. De Wet van Zyl

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Abstract

The purpose of this study is to investigate specific contributing factors affecting quality assurance in the diagnosis of conventional cervical smears. More than half of South-African women fail to have one cervical smear in their lifetime and +/- 50% of those who do have cervical smears taken, are lost to follow-up.

Since cervical cancer is the most common malignancy amongst women in developing countries, the medical profession will have to endeavor to screen a higher rate of women and ensure a 100% quality assurance with every patient treated in order to reduce the unacceptable high incidence of cervical carcinoma. At this stage it seems like an impossible task to screen all women in South Africa, due to far-off rural areas, shortage of medical professionals and the lack of knowledge of some women of the necessity of cervical smears.

Many newly qualified South-African doctors leave the country to go and work elsewhere. South Africa then in turn has to recruit doctors from other countries to staff the State hospitals and clinics. Some areas have one doctor to thousands of patients, insufficient nursing personnel and inadequate equipment. Doctors in some areas cannot cope with the volume of work and the long hours. One has to accept that the quality of the management of some patients is affected negatively.

There are a number of medico-legal issues (world wide) in relation to aspects of cervical cancer prevention practices which are controversial and are of particular concern to all of those involved in cervical cancer prevention. Various countries have therefore formed different national organizations to address the medico-legal issues in screening for the prevention of cancer. These organizations monitors procedures, internal quality control as well as external quality control.

In South Africa, medico-legal cases are not so prevalent, but may become so shortly. The South-African medical professionals therefore have to ensure that their quality of work

conforms to accepted good practice in all circumstances. State hospitals serve thousands of patients per month and it is an every day occurrence to see long queues of patients sitting waiting for doctors and who often have to come back the following day. The situation appears to be much improved in private practice and since patients have medical cover and accessible medical facilities.

Since cervical cancer is the most common malignancy amongst women in developing countries, the medical profession will have to endeavor to screen a higher rate of women and ensure a 100% quality assurance with every patient treated in order to reduce the unacceptable high incidence of cervical carcinoma. At this stage it seems like an impossible task to screen 100% of women in South Africa, due to far-off rural areas, the shortage of medical professionals and ignorance of patients.

Quality assurance is therefore of paramount importance to every medical professional for every patient treated.

Laboratories all worldwide have been, or are in the process of being accredited by their specific accreditation authorities. The main reason for this is improvement of quality control and therefore quality assurance.

The South African National Accreditation Society (SANAS) now accredits various laboratories in South Africa with the view of accreditting all laboratories within a certain time limit. The Ampath laboratory Port Elizabeth was successfully accredited during 2001. Accredited laboratories have to uphold a very high degree of quality to remain accredited. A team of professionals inspects the laboratory every 2 years and other quality assurance staff inspects the laboratories every few months. All aspects of the laboratory are checked, e.g. the qualification of staff, their registration with the Health Professions Council of South Africa (HPCSA), their curriculum vitaes, equipment, safety of the laboratory etc. etc.

Since the laboratory chosen for this study, is accredited, the author evaluated every cervical smear that was received in the laboratory since the year 2000, with the following objectives in mind:

- Whether the presence or absence of an endocervical component has an effect on the adequacy of cervical smears
- To determine the effect of using smaller coverslips on quality assurance in the cytology laboratory
- Evaluate the effect that manual re-screening of smears has on quality assurance in the cytology laboratory.

As there is a shortage of cytotechnologists and pathologists worldwide, several countries make use of automated screening devices as primary screening or secondary screening for quality assurance. These devices were tested in some laboratories in South Africa but were found to be very expensive and sensitivity and specificity were not up to standard. Sensitivity is a measure of the ability of a test to detect the abnormal - Sensitivity is the ratio of true positives to true positives + false negatives. Specificity is a measure of the ability of a test to correctly identify the negative - Specificity is the ratio of true negative to true negatives + false positives. The automated screening machines failed to identify abnormal cells amongst inflammatory cells, as well as in very blood stained smears. Several other problems also occurred and an increasing number of smears had to be manually rescreened, thus making this exercise costly and not helpful as a quality assurance instrument.

The slides used for this thesis, have been retrieved from the archives of the Ampath laboratory in Port Elizabeth.

Fourteen specific contributing factors affecting quality assurance in the diagnosis of cervical smears are also discussed and conclusions and recommendations given.

Acknowledgements:

I wish to thank everyone sincerely who made this study possible, especially:

- Dr Nanette Smith as well as Dr de Wet van Zyl for all the useful input and continuing support in compiling this thesis. I know you both have very demanding schedules.

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- To my family, Leon, Duane and Shandre – Thank you very much for all your patience! I am sure to see more of you now!

LIST OF ABBREVIATIONS:

A

Abn	**Abnormal**
Abs	**Absent**
AGC	Atypical glandular cells
AGUS	Atypical glandular cells of uncertain significance
ALTS	ASC-US/LSIL Triage study
ASCCP	American Society for Colposcopy + Cervical Pathology
ASC-H	Atypical squamous cells a HSIL cannot be excluded
ASC-US	Atypical squamous cells of uncertain significance
ASC-US – SIL	Ratio of Atypical squamous cells of uncertain significance to cells from Squamous intra-epithelial lesions

C

CAP	**College of American Pathologists**
CCHSA	Canadian Council on Health Services Accreditation
CIN	Cervical intra-epithelial neoplasia
CIN 2	Cervical intra-epithelial neoplasia – grade II (HSIL)
CIN 3	Cervical intra-epithelial neoplasia – grade III (HSIL)
CIS	Carcinoma-*in-situ* **–** (HSIL)
Comp	Endocervical component
CPA	Clinical Pathology Accreditation
Cx	Cervix / cervical

F

FINAS	**Finnish Accreditation Service**
FNA	Fine needle aspiration

H

HPCSA	Health professions council of South Africa
HPV	Human papilloma virus
HSIL	High grade squamous intra-epithelial lesion

I

IAF	**International Accreditation Forum**
IEC	**International Electrotechnical Commission**
ILAC	**International Laboratory Accreditation Cooperation**
ISO	**International organization for Standardization**
IUCD	**Intra-uterine contraceptive device**

J

JCAHO	Joint Commission on Accreditation of Healthcare Organizations

L

LAP	**Laboratory accreditation program**
LSIL	Low grade squamous intra-epithelial lesion

N

NATA	National Association of Testing Authorities
n/c	Nuclear to cytoplasmic ratio
Neg	Negative for malignant cells
No	Number
Norm	Normal

P

Pres	**Present**

Q

QA	**Quality assurance**

S

SANAS	South African National Accreditation Society
SHE	Safety, Health and Environment
SOPs	Standard Operating Procedures
Sm	Smear(s)
SWEDAC	Swedish Accreditation

T

TELARC	Testing Laboratory Registration Council
Trans	Transformation zone

U

UK	United Kingdom

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CHAPTER 1

INTRODUCTION

What is cytology?

In pathology, cytology refers to the study under a microscope of individual cells to establish a cause of disease, most commonly for premalignant or malignant conditions. Cells are stained with a number of tinctorial or other stains that enable the nuclear and cytoplasmic features of cells to be examined.

The cells that are examined in cytological samples usually originate from epithelial, or epithelial like tissues, and are most simply obtained as the epithelium exfoliates. These cells may be naturally exfoliated as in sputa or urine, or the cells may be mechanically obtained by brushing or scraping.

Sometimes cells may be obtained by the use of fine needle aspiration (FNA) that enables more deep-seated tissues that would not necessarily exfoliate, to be sampled.

Cytological specimens are usually divided into gynaecological and non-gynaecological samples. The former are from women within cervical screening programs, the latter generally being diagnostic samples from patients with suspected disease.

Cervical smears form part of gynaecological (Fig. 1) cytology. Normal cell types that may be seen within a cervical smear include epithelial cells from the ecto- and endocervix, the vagina and endometrium.

Inflammatory cells, red blood cells, fungi and viral cytopathic effect may also be seen (Geisinger, 2003).

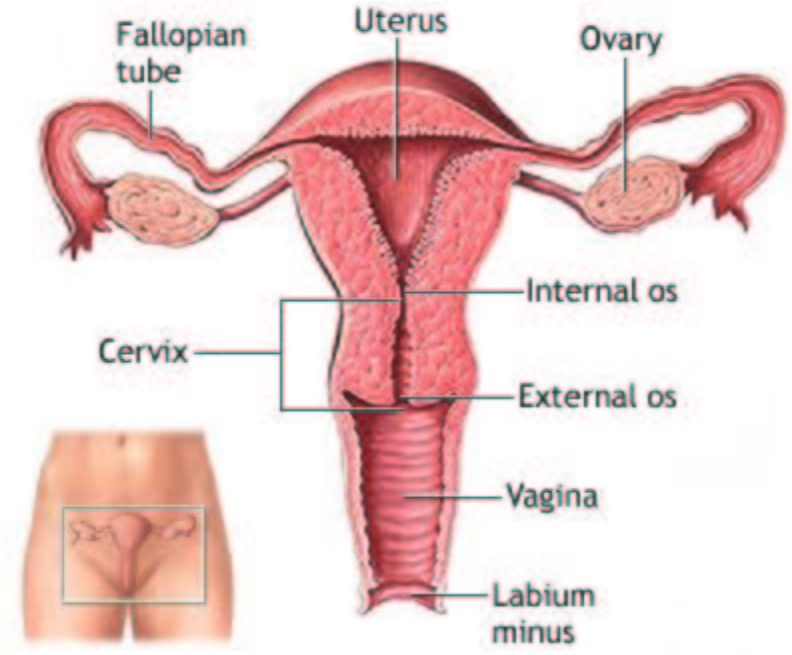


Figure 1: Female genital tract (Taken from http://www.nlm.nih.gov/medline)

What is the problem?

Only 41% of women in South Africa present for cervical sampling "some or other time in their lives"!! Of these, less than 50% follow their recommendations as per health professional, doctor or pathologist instructions. Therefore, the diagnosis of ASC-US (Atypical squamous cells of uncertain significance) or even LSIL (Low grade squamous intra-epithelial lesion) may not have any impact on the patient and she may abscond for the rest of her life.

These patients may be lost to follow-up and the ideal situation would be for them to wait for their results and be treated or treatment recommended at the initial consultation (Cross, 1997). According to South African government legislation, a woman is entitled to go for cervical smear screening 3 times during her lifetime. Private patients and even those with medical aids seem to go for cervical smears as required, i.e. pre-natal or post natal, with irregular bleeds etc. (Sankaranarayanan, 2003).

Any family practitioner or internist etc., when seeing female patients for non-gynaecological problems, should not miss the opportunity to obtain a cervical smear whenever appropriate (Dudding, 1995).

Since cervical cancer is the most common malignancy amongst women in developing countries, the medical profession will have to endeavour to screen a higher rate of women and ensure a 100% quality assurance with every patient treated in order to reduce the unacceptable high incidence rate of cervical carcinoma. At this stage it seems like an impossible task to screen 100% of women in South Africa, due to far-off rural areas, the shortage of medical professionals and ignorance of patients.

Many newly qualified South-African health professionals leave the country to go and work elsewhere. South Africa then in turn has to recruit health professionals from other countries to staff the State hospitals and clinics. Some areas have one doctor to thousands of patients and insufficient nursing personnel and inadequate equipment. Health professionals in some areas cannot cope with the volume of work and the long hours. One has to accept that the quality of the management of some patients is affected negatively (own observation).

What is the solution to the problem?

Although various authorities have been discussing quality assurance globally since the mid-eighties, accreditation of medical laboratories only became compulsory in 1996. The necessity for compulsory accreditation was the result of medico-legal cases in different countries, concerning false-negative cytological results. In South Africa, medico-legal cases are not so prevalent, but may become so shortly.

Quality assurance is therefore of key importance in the evaluation of cervical smears (Benedict and Murphy, 1985). Quality assurance applies to all clinical and non-clinical staff involved and therefore it seems worth the money and trouble to train every member of staff that we have access to and make them aware of the statistics and the absolute importance of QUALITY ASSURANCE (Faulkner, 2003).

What is quality?

The concept 'quality' can be described in many ways. According to the international standard ISO 8402, 'quality' is a totality of characteristics of an entity that bear on its ability to satisfy stated and implied needs. The achievement of satisfactory quality involves all stages of the quality loop as a whole (Linko, *et al*., 2001).

The operation of laboratory medicine in patient care, monitoring and diagnosis is strongly linked to measurements and observations applied to laboratory samples. Producing reliable results within a reasonable turnaround time is the ultimate responsibility of medical laboratories. The total quality of the laboratory service, from the pre-analytical phase through the analytical phase to reporting, is to support the clinicians' decision making.

The proper management of laboratory processes needs supervised personnel doing the right things in the right way. Well-defined rules are necessary for this management, as the numerous processes range from sample taking to reporting (Linko, *et al*., 2001).

International standards, guides and legislation support the establishment and implementation of quality systems. The quality of laboratory results, as being the end products of the process, thus strongly reflects the internal efficiency and the outcome of quality assurance (Linko, *et al.,* 2001).

1.1 Contributing factors affecting quality assurance in the diagnosis of cervical smears:

The following procedures should be tested and medical professionals informed about these measures. These individuals should be monitored and statistical feedback provided in order to audit their extra training (Faulkner, 2003).

1.1.1. Taking of smear:

Collection of a cervical cytology specimen (Fig. 2) is usually performed with the patient in the lithotomy position (patient on her back with hips and knees flexed and the thighs separated).

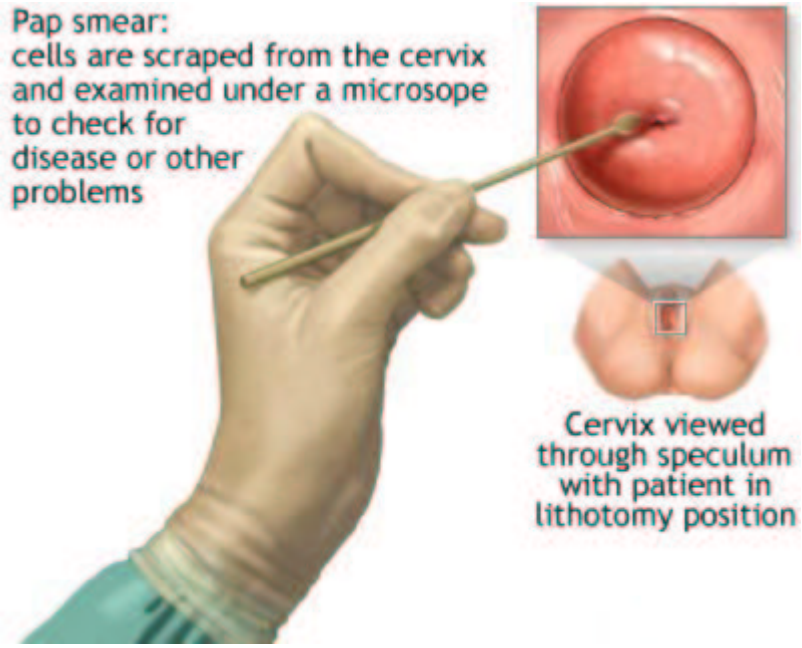


Figure 2: Illustration of taking of cervical smear (Taken from http://www.nlm.nih.gov/medline)

A sterile or single-use bivalve speculum of appropriate size is inserted into the vagina without lubrication, since lubrication materials may obscure or otherwise negatively affect the cells. Warm water may be used to facilitate insertion of the speculum. The position of the speculum should allow for complete visualization of the cervical os and ectocervix. The entire vagina is also checked for any ulcers or suspicious areas (Fig. 3).

There are various ways of taking cervical, vaginal and vault smears (Kieswetter, *et al*., 2001). Various devices are used according to the clinician's choice or the finances available (Valenzuela, 2001).

The crucial point is to be able to visualize the area, to take a satisfactory sample (including transformation zone if applicable) and then to transfer most of these cells on to the glass slide. The main source of false negative diagnoses is the insufficient gaining of material from the endocervical canal (Geisinger, 2003).

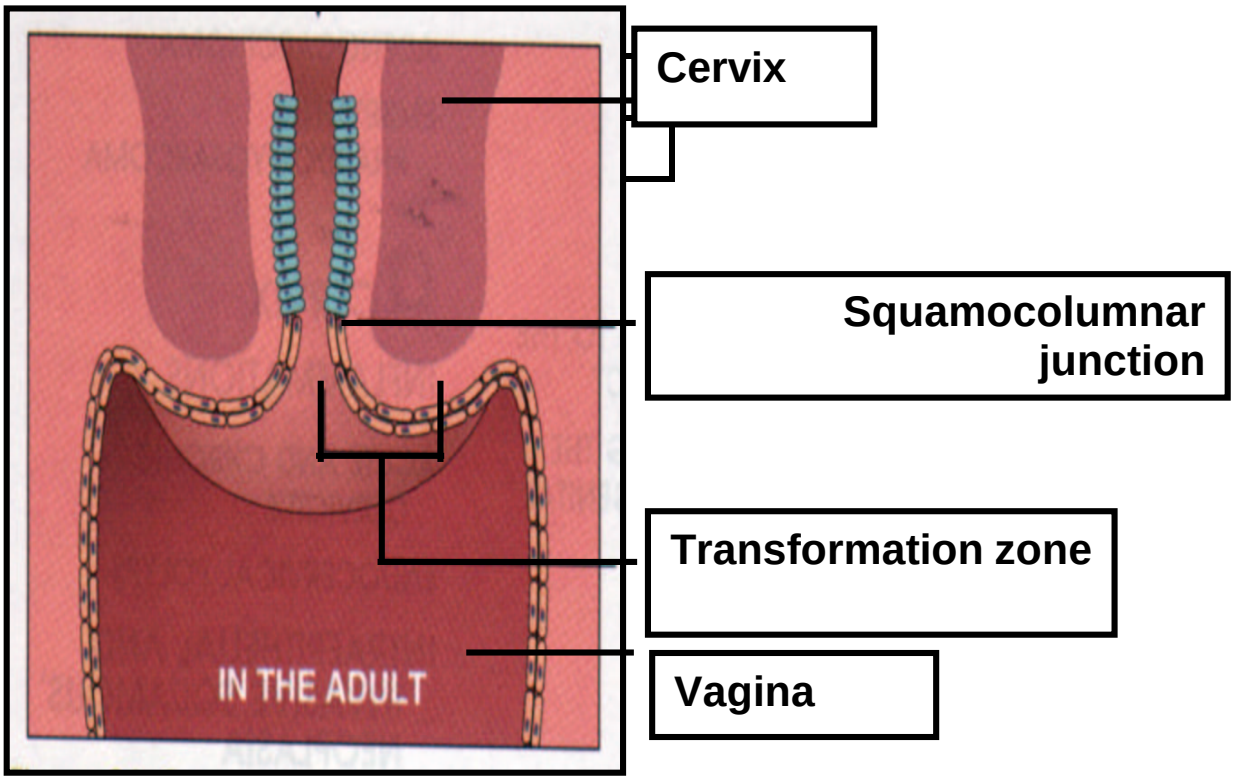


Figure 3: Transformation zone (Taken from The Bethesda System)

Various spatulas are available to obtain cells from the cervix and the application of cells onto a glass slide. Some health professionals prefer wooden spatulas and others the cyto-brush. The main concern is to get an adequate smear from the correct area of the ecto- or endocervix, post-fornix or vaginal wall (Diag. 1).

1.1.2. Fixation of smear:

The fixation of a smear is very important. Poor fixation makes cells unsuitable for cytological evaluation. All health professionals are supplied with cans of aerosol spray-fixative, which is alcohol and carbowax based (SOP-LP-PCYTO-013).

1.1.3. Marking of the slide:

The smear should immediately be marked with a pencil or diamond pen on the frosted end of the slide to accurately identify the slide (SOP-WI-PCYTO-001).

1.1.4. Submission of clinical data:

The accompanying form should contain all relevant details pertaining to the patient. Clinical details are very important to the cytologist as this can explain the hormonal status, of the cells on the smear (e.g. atrophy in estrogen deficiency), blood (e.g. during menstruation), and the presence or absence of endometrial cells (e.g. pre- versus post menopause), (fig.4), infection, etc. The patient's age and contraceptives used, aids in correct interpretation of the cells on the smear (Marques and Mc Donald, 2000).

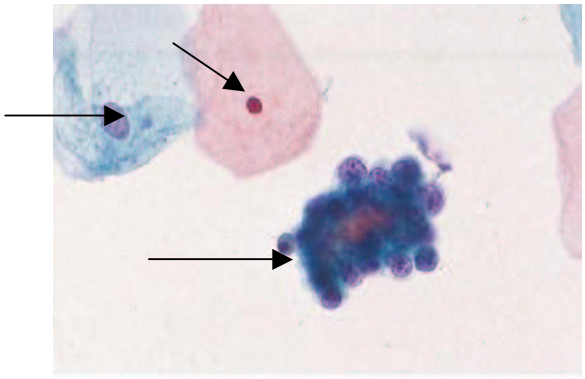


Figure 4. Example of cervical smear with isolated squamous cells and a group of endometrial cells. ? Menstrual smear. Three-dimensional cluster of endometrial cells.
(Note – the blue cell with the vesicular nucleus is an intermediate type cell and the pink cell with a pyknotic nucleus is a superficial type cell – see page 10).

(Taken from The Bethesda system for Reporting cervical cytology).

1.1.5. Transportation of cervical smears:

Timeous Cytoprep-fixation is the perfect "transport medium" for a conventional cervical smear and this fixation will keep the cells in perfect condition for a couple of days (SOP-LP-PCYTO-013).

1.1.6. Receiving the specimen in the laboratory:

Training on the receipt of specimens in the laboratory is regarded as of utmost importance as NO mistake may be allowed here. Only one specimen is opened at a time

with its corresponding form. The information on the slide is compared with those on the form, and if correct, the slide is assumed to belong to that form and both the slide and the accompanying form are allocated a unique laboratory number. This number is also entered in the daybook and all the relevant data is written next to this number. This slide is now ready to be stained (SOP-WI-PCYTO-001).

1.1.7. Staining of slides:

Before any diagnosis can be made on any slide, the slide needs to be stained by various stains to enable the screener to interpret the cells with the aid of a microscope. Laboratories vary in their way of staining cervical slides since some procedures are automated with different pre-set staining machines. Other laboratories stain manually.

The stain differentiates superficial from intermediate squamous cells and generally leaves mucin unstained. The main components of the stain are haematoxylin which stains the nuclei, and OG6 and EA65 which stain the cytoplasm. The cytoplasmic dyes behave as a trichrome stain which relies upon the interaction between the dye molecule size and tissue pore space. The rationale of the stain is based on the fact that dye molecules are displaced by molecules of increasing size until the largest dye sits in the largest pore space.

Quality control of staining of slides is of utmost importance and the quality of staining of every slide is commented on, on every worksheet. Corrective action is taken accordingly. The filtering of the contents of the staining dishes is also very important as this avoids contamination of one batch of smears by the other (SOP-WI-PCYTO-008).

1.1.8. Mounting:

The slide is mounted manually with a mounting medium and a glass coverslip placed over the mounting medium to protect the smear and ensure that the slide can be kept for a long period of time.

All stained slides are mounted under an extractor fan using the approved mounting medium, e.g., Entellan or DePex. Cross-contamination (by touching the smear with the pipette and then applying the mounting medium onto another slide) and air-bubbles are avoided at all times – and also commented on, on every worksheet. Take note that an air

bubble can obscure an abnormal cell (SOP-WI-PCYTO-010). Certain smears can have thicker areas where the smear has not been spreaded evenly. It can then be difficult for the mounting medium to cover these thick areas. This causes air-bubbles and the slide has to be remounted.
By law, all abnormal smears must be retained permanently – therefore the quality of the mounting medium must also be assessed, as the quality of the staining has to be maintained for future reference. Should the staining intensity fade, the slide can be placed in xylene until the coverslip comes loose and the mounting medium can be dissolved, and the slide restained for further use (SOP-WI-PCYTO-010).

Most Cytology laboratories that evaluate high volumes of slides per week make use of an automated mounting machine. These machines can mount up to 400 slides per hour and have different settings for the various sizes of coverslips used in that specific laboratory.

The size of coverslips used is a very important component of quality assurance, as pathology laboratories do differ on the percentage of abnormal cells missed upon using a smaller coverslip (Oommen, *et al*., 1998).

1.1.9. Labelling:

Every slide has to be labelled with a unique laboratory number stating clearly the current year and the department, e.g. 04CS00001 (04 for the year 2004, CS for the cytology department and 00001 being the numerical number). This will enable the cytologists to retrieve slides in years to come.

Before a slide is labelled, the name and number on the slide as well as the form is compared and the appropriate label is attached to the frosted end of the slide. This is the third quality assurance check which ensures that the slide has the correct name, number and label (SOP-WI-PCYTO-013).

1.1.10. Screening:

Screening entails looking at every cell on the smear and evaluating the cells through a microscope. Examination of the nucleus of the cell helps to diagnose abnormality and examination of the cytoplasm of the cell helps to interpret the exact origin of the cell.

The normal cell-types that may be seen on a cervical smear sample may include epithelial cells from the ecto- and endocervix, the vagina and endometrium. In addition acute and chronic inflammatory cells, red blood cells and spermatozoa may be seen.

Non-keratinised squamous cells reflect the hormonal status of the patient that will be related to her age, the use of hormonal contraceptives or hormonal replacement therapy, and the stage of her menstrual cycle.

Superficial squamous cells are shed from a fully mature squamous epithelium, which has developed its full thickness under the influence of oestrogen and will be most numerous at the middle of the menstrual cycle. The cells stain pink with the Papanicolaou stain, are angular outline, 40-60 micrometers in diameter, and contain a shrunken hyperchromatic pyknotic nucleus.

Intermediate cells are shed from the surface of semi-mature epithelia that shows a diminished response to oestrogen or the effects of progesterone and are commonly seen at the latter stages of the menstrual cycle, they are 30-60 micrometers in diameter, contain glycogen and stain blue/green with the Papanicolaou stain and contain a clearly defined round or oval vesicular nucleus 8 micrometers in diameter.

Parabasal and basal cells are seen in the absence of either oestrogen or progesterone, and are found in pre-pubertal smears, post-menopausal smears or post-partum smears. The cells are small, 15 -20 micrometers in diameter, sometimes in syncitial sheets, with vesicular nuclei. They usually stain blue and contain a regular granular nucleus with an occasional chromocentre. Cells exfoliated spontaneously appear round whereas those detached with the spatula appear as sheets or have cytoplasmic processes.

Squamous metaplastic cells arise when the columnar endocervical cells are exposed to the acid environment of the ectocervix. In response the reserve cells within the epithelium

form squamous cells rather than columnar cells, the effect of which is to move the squamous-columnar junction towards the ectocervix. Immature squamous metaplastic cells show nuclear and cytoplasmic features of both columnar and squamous cells. It is during the process of transformation that the cells become susceptible to the HPV infection that may cause them to develop pre-malignant changes.

The sample should be taken from the squamo-columnar junction and should contain both squamous cells and columnar cells from the ecto- and endocervix, squamous metaplastic cells may also be present (http://www.curran.pwp.blueyonder.co.uk).

At the Ampath laboratory in Port Elizabeth, conventional cervical smears and normal manual light microscopy are used. (Most laboratories in South Africa use conventional cervical smears and light microscopes). With hundreds of thousands of cells on every slide, the screener depends heavily on quality assurance steps 1 to 9, as this will make her/his job easier!

The slide is usually screened from the labelled side, up and down the width of the slide, to the end of the glass coverslip. Every cell must be evaluated correctly and cautiously and reported on in a specific way acceptable and understandable by everybody concerned. At this stage, the Bethesda System 2001 is in use. This system has preferred nomenclature and recommendations (Apgar, *et al.*, 2003).

The screener checks on the previous records of every patient and any previous results or slides are retrieved for reviewing. These findings are then compared to the current slide on this patient (SOP-WI-PCYTO-035).

Any cell that is worrisome to the screener is marked with a permanent marker and marked as such at the bottom of the worksheet. The slide and worksheet is then handed to the senior checker. The slide is then rescreened by the checker and discussed with the screener. An opinion is formulated and all abnormal slides are then presented to the pathologist in charge, after which a final diagnosis and recommendation is generated (SOP-WI-PCYTO-035).

In the 1990s, it was in vogue to develop computers attached to the microscope, which would support the cytotechnologist with conventional microscopy. The system

connected sensors to the microscope stage to a computer and monitor display. This provided a map of which areas of the slide had been screened, useful when training in microscopy techniques and in ensuring the complete screening of slides by cytotechnologists. The device offered feedback to the cytologist and laboratory managers on screening techniques. Quality control data including time spent per slide, percentage coverage of the slide surface and total workload was also collected and analyzed. Unfortunately these systems were costly and perceived as having only quality and cytotechnologist support value and have not been commercially successful. Most are no longer developed (Gray and McKee, 2003).

1.1.11. Typing:

The report is typed and handed to the pathologist. All relevant data is rechecked before the result is signed off and ready to be printed. This result is now ready to be posted or e-mailed (SOP-WI-PHIST-500).

1.1.12. Re-check:

Although the result is now ready and available to the referring clinician, (in our laboratory) EVERY smear is rescreened by the checker to ensure a 100% correct result. Once again the patient's history is checked, and any possible previous reports retrieved and checked according to the current slide and follow-up recommended (SOP-WI-PCYTO-035).

If a recheck shows a difference in opinion, that slide and form is submitted to the pathologist in charge, discussed with the screener and monitored as such. Amendments and recommendations may be authorized by the pathologist in charge (Baker, *et al*., 1995).

When a result is signed off, the patient's details, doctor's details, clinical details, as well as the complete report are double checked as an extra quality assurance measure (SOP-WI-PCYTO-035).

1.1.13. Statistics:

The statistics of every screener is monitored (Fig. 5). This entails the number of slides screened by each individual screener per day as well as their percentage rate of agreements and disagreements. Minor and major mistakes in screening are managed according to SOP-WI-PCYTO-037. Minor mistakes are regarded as the misinterpretation of the presence or absence of endocervical cells, fungi, etc. resulting in no change to the management of the patient. A major mistake is regarded as misinterpretation abnormal cells which results in a change to the management of this patient, e.g. for a diagnosis of LSIL the management of the patient is colposcopy, but for a diagnosis of HSIL (High grade squamous intra-epithelial lesion) the management of the patient is colposcopy as well as endocervical assessment. Every cytology laboratory endeavours to a 100% accuracy rate, but unfortunately that is not always possible.

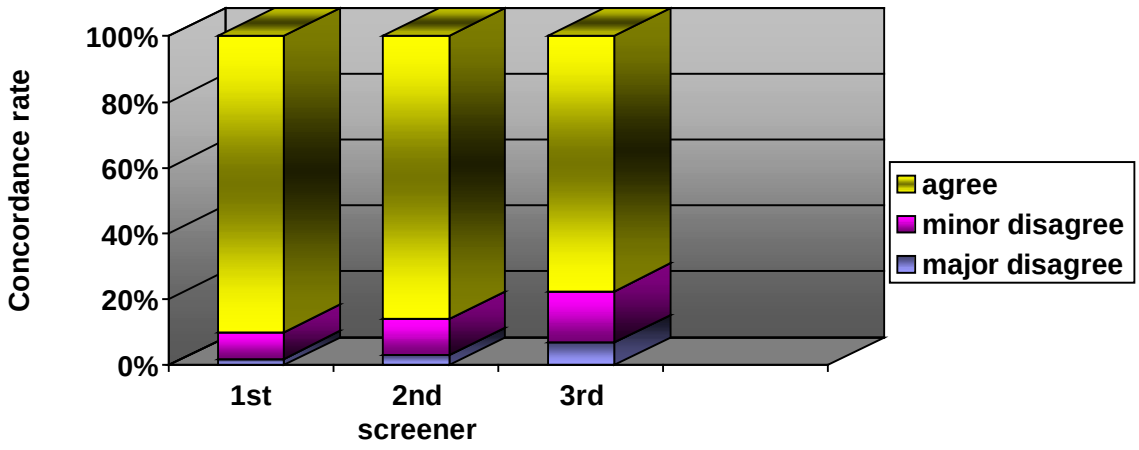


Figure 5: Screener averages for the month of October 2004:

(Data taken from Ampath laboratory Port Elizabeth)

The turnaround-time of all cytology specimens is monitored. The acceptable turnaround-time according to SANAS is 5 working days. The cytology laboratory at Ampath Port Elizabeth has a turnaround-time averaging 6 hours. Turnaround-time is calculated as the time from where the specimen arrives in the laboratory, to the time the result is signed out and posted (pre-analytical, analytical and post-analytical phases).

1.1.14. Comparison with histology specimen:

In histopathology, disease states are diagnosed by studying stained tissue sections of biopsies, body tissue organs and the diagnosis are based on the pathologist's acquired knowledge and experience (Fig. 6). This can often lead to difference of opinion and assessment (Williams, *et al*., 2003).

Despite this, histology is still regarded as the gold standard. Similarly, in cytopathology diagnoses are made on stained smears and body fluids and the diagnosis is based on the cytotechnologist's and pathologist's acquired knowledge and experience. This can also often lead to difference of opinion and assessment (own observation).

All cervical biopsies are therefore checked for previous cervical smear results, and *visa versa*. When the results differ, the appropriate action is taken by the pathologist and discussed with the submitting doctor (SOP- WI-PHIST-096).

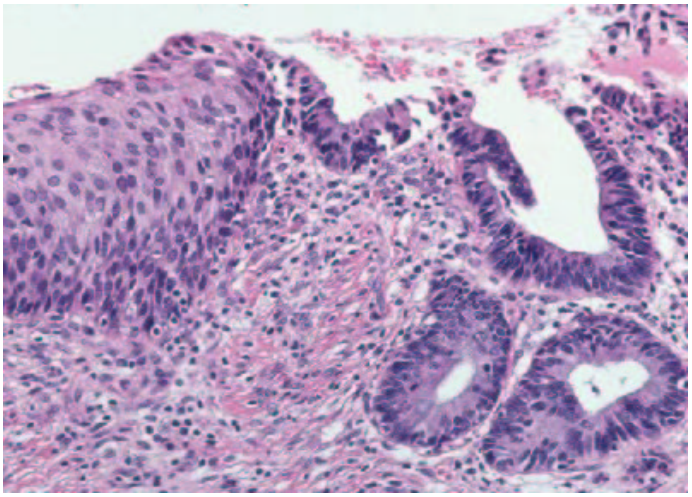


Figure 6: A slide prepared from a histology section.
(Taken from The Bethesda system for reporting cervical cytology).

Glandular and squamous lesions may coexist. In figure 7, HSIL is seen on the squamous epithelial surface on the left side of the image, and the endocervical adenocarcinoma *in situ* is present in the gland spaces on the right. (Adenocarcinoma *in situ* is a non-invasive high-grade neoplasm of glandular cells – a precursor to Adenocarcinoma).

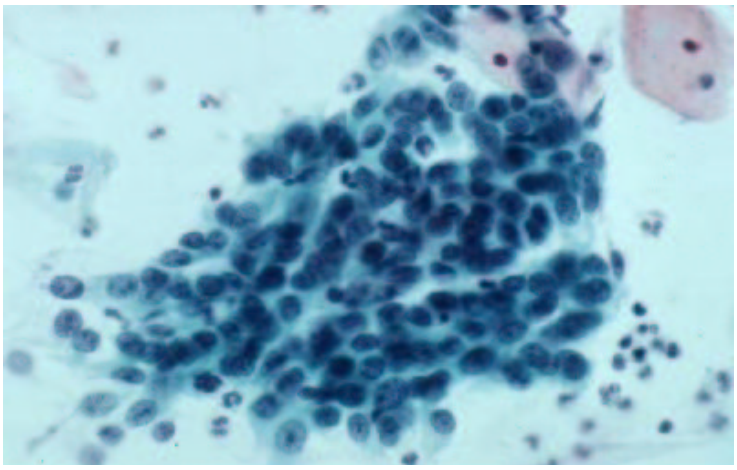


Figure 7: Cervical smear taken from 42-year old woman. (HSIL)
(Taken from The Bethesda system for reporting cervical cytology).

Although uncommon, nucleoli may be seen in HSIL. The chromatin appears less coarsely granular.

Figures 6 and 7 shows the difference in screening a histology slide as opposed to a cytology slide. Figure 6, the histology slide, shows the growth pattern of the cells. Figure 7, the cytology slide, shows the cells exfoliated or scraped off when the smear is taken.

1.2 Statement of the problem:

The question arises whether we should aim to screen a greater percentage of women in South Africa or whether (taking the financial constraints into consideration) we should rather endeavour to increase the quality of every diagnosis on EVERY cervical smear on the 41% of women we do receive.

The question arises why patients present with advanced cervical disease *ab initio* in countries where cervical screening programs are available. This is regarded as a failure of cervical smear screening.

There are a variety of interrelated factors relating to failure of cervical smear screening, these include:

Patient –related factors:

A large group of problems related to failure of cervical smear screening are patient – related errors, including women who do not go for cervical smears at all or those that are loss to follow-up (Golden and Hickman, 2004).

Other patient-related factors occur (Howe, 2003). For example, douching or coitus may mechanically remove the superficial cell layers that the cervical smear samples, resulting in false-negative smears due to sampling errors.

Patients may also delay seeking medical attention even when they have symptoms that they know are suspicious, such as abnormal vaginal bleeding (De Paula and Madeira, 2003).

In addition, many things known to be important risk factors are under the patient's control, such as smoking, age at first intercourse, number of sexual partners and method of contraception (Karube, *et al*., 2004).

Clinical errors:

The next group of errors, unfortunately, comes from those who should "be informed," the health care providers and clinicians. Among the most common errors are failure to take cervical smears at all, or failure to take a representative cervical smear – a thinly spread, well-fixed smear containing both ecto- and endocervical component. The smear must be taken under direct visualization and with considerable pressure.

Failure of the clinician to provide pertinent clinical details may compromise cytological interpretation of the smear. Other common clinical errors include failure to follow-up abnormal smears, failure to perform a biopsy of suspicious lesions, and failure to investigate suspicious clinical symptoms. Unfortunately, it also seems likely that some clinicians accept a negative report at face value, disregarding statements of specimen adequacy. Miscellaneous problems, such as clerical errors, also occur (De May, 1995).

Cytopreparation and diagnostic errors:

Errors in diagnosis can occur in relation to cytopreparation, screening, or interpretation, as well as to general laboratory problems, such as mislabelling slides.

There are several points to be considered in assessing screening errors, i.e. failure to detect abnormal cells that are present on the slide. Three of the most common diagnostic problems relate to the presence of few abnormal cells, small abnormal cells and bland abnormal cells. Other problems in interpretation include inflammatory changes, regeneration/repair, radiation effect, and atrophy. Another diagnostic error is rendering a negative diagnosis on unsatisfactory material.

There is also a class of cells that have been designated "litigation cells." (And with the filing of criminal charges in cases of cervical smear misdiagnoses, these cells may well

become known as "jail cells.") Litigation cells are important sources of false negative as well as false-positive results. The clinical significance of litigation cells ranges from completely benign to frankly malignant. They are small, medium, or large cells, with low, intermediate, or high n/c (nuclear to cytoplasmic) ratios, smooth or irregular nuclear membranes, fine to coarse chromatin, which can range from pale to dark, and have inconspicuous to prominent nucleoli (De May, 1999).

Lesion-related errors:

For various reasons, some lesions fail to exfoliate cells in numbers sufficient for diagnosis (Gupta and Sodhani, 2004). Small or inaccessible lesions may be difficult to sample adequately, and therefore false-negative cytology is more common in smaller lesions (Coldman, *et al*., 2003).

Unfortunately, smears are more often inadequate in patients with severe epithelial abnormalities. Necrosis, inflammation, or bleeding may obscure, alter or dilute the abnormal cells, making diagnosis difficult (De May, 1999).

Rescreening and quality assurance in the laboratory is of utmost importance (Hadwin, 2003).

1.3 Objectives:

As these are just some of the aspects of normal daily routine in cytology practice that can contribute to a far better abnormality pick up rate cytologically, the author has decided to take the information in the laboratory and prove the positive influence of proper quality assurance on correct diagnoses to which every patient is entitled. The findings will involve many patients as an average of 4600 cases of cervical cancer was reported in 2002.

Taken that this figure only involves the 41% of women that go for cervical smears, the author was interested to know what the figure would be with proper quality assurance at all levels.

The objectives are thus:

- To determine whether the presence or absence of endocervical component has an effect on the adequacy of cervical smears
- To determine the impact of using smaller coverslips on quality assurance
- Evaluate the effect that re-screening of smears has on quality assurance in the cytology laboratory.

1.4 Methodology

The (n = +/- 61 000) cervical smears received in the laboratory since the year 2000 will be evaluated and re-evaluated with the above objectives in mind. The slides will be retrieved from the archives of the Ampath laboratory in Port Elizabeth.

CHAPTER 2

Literature overview

2.1 Quality assurance in cytology: an overview

Over the last 15 years, cervical screening using the conventional cervical smear test has been introduced successfully in many countries of the world (Hudson, 1985). Until the 1990s, the accuracy of the cervical smear had rarely been questioned although its efficacy has never been tested in a prospective blinded trial. The general public and most health care professionals failed to recognize the fundamental limitations of the cervical smear as a screening test and expect standards that even most diagnostic tests cannot achieve.

If reductions in the incidence and mortality of cervical cancer are to be achieved, attention has to be focused on the screening test itself (Kurtycz, 2003). Notwithstanding the effectiveness of the cervical smear, there is good evidence that the smear test is not

sufficiently sensitive, specific, and consistent in reliably detecting the presence of cervical intra-epithelial neoplasia (CIN) in the cervix (Hoffman, *et al*., 2003).

In the literature, the sensitivity of a single cervical smear is assessed at around 50-60% (Hoffman, *et al*., 2003). This is perhaps not surprising since, unlike most other laboratory tests, the cervical smear methodology has remained essentially unchanged in over 60 years.

The method of collecting and preparing a cervical sample has inherent limitations, therefore the high inadequate rates and low sensitivity can be overcome by repeating the test at regular intervals.

The deficiencies of conventional cervical smears are due to many factors, including the use of wooden samplers that are cheap and designed to collect material from the cervix but are not flexible or designed to spread material evenly onto a glass slide (Fig. 11).

The sampler used by the clinician is basically of his/her own choice, but the point of decision should be diagnostic accuracy rather than a high proportion of good quality smears.

In addition to variable dexterity in collecting and preparing the sample, smear takers use a multiplicity of different fixatives in a variety of different ways. Thus there are great variations in the quality of smears sent to the laboratory: cellular distribution on the glass slide is uneven; cells may be obscured by blood or inflammatory exudate or thick streaks of other cells; total numbers of abnormal cells may be very few; fixation is variable and partial drying artefact is common, leading to poor staining and loss of microscopic detail. Not only do all of these influence the adequacy of the sample, they also limit the ability of the laboratory staff to interpret the cells present on the smear correctly. Sometimes the

result is just too few intact cells on the slide, particularly in the presence of atrophy or cytolysis, rendering it inadequate for assessment (Gray and McKee, 2003).

It is worth noting that abnormal cells are not collected evenly on the sampling device and only a selected proportion of the cells are placed on the glass slide. In fact, the amount of material transferred successfully to the glass slide ranges from around 60% at best, to less than 10% at worst and some samplers have more of a trapping effect than others. Thus, depending on the sampler used, up to 90% of the material scraped from the cervix may be discarded with the sampler (Apgar, *et al.*, 2003).

Furthermore material is transferred from the sampler to the slide in a non-randomized way and therefore the cellular material on the glass slide is spread unevenly. Abnormal cells may be present only in one small area, rather than evenly distributed across the slide. *Contrary to public belief, sampling and preparation together are responsible for about two-thirds of false negative results* (Gray and McKee, 2003).

The low sensitivity of a single cervical smear is therefore mainly due to incorrect or inadequate sampling of the cervix; poor transfer of cells to the glass slide; sub-optimal preparation and fixation and only to a lesser extent, the microscopic assessment in the laboratory. However, all of the above affect the ability of the cytotechnologist to identify abnormal cells on the slide. Only a small proportion of all false negative tests are due to human error in microscopic assessment of the laboratory (Fig. 8).

The following chart shows the difference in opinion of three qualified cytotechnologists.

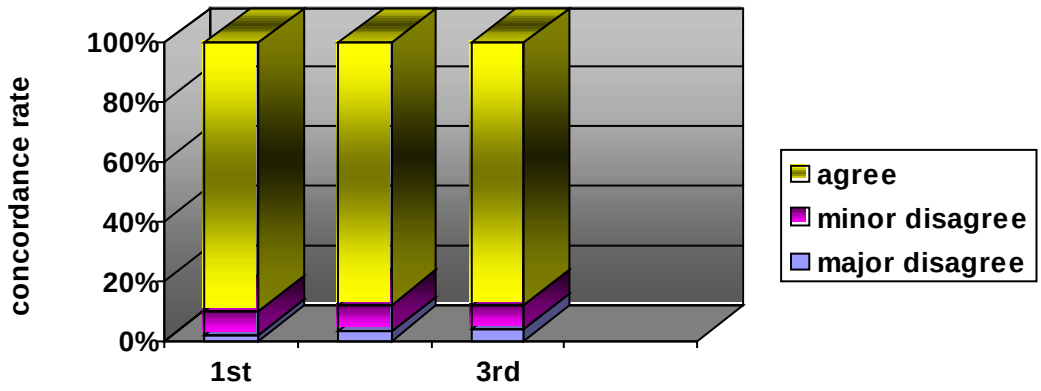


Figure 8: Difference in opinion of screeners for the year 2003

(Data taken from averages of screener opinions for the year 2003 Ampath laboratory Port Elizabeth).

The false positive cervical smear is far more prevalent and far more costly to the health-care system and causes emotional stress for women.

The move towards new technologies is driven by the need to improve these deficiencies in the current methods of cervical screening. Improvements in diagnostic accuracy of the cervical smear need to begin in the doctor's rooms with improved techniques of specimen collection to allow better quality samples and better slide preparations. If a representative sample is not removed and sent to the laboratory, there is little that laboratory staff can do to improve the sensitivity of the test.

Thus the quality of the sample sent to the laboratory, the presentation for microscopic assessment and the accuracy of the observer need to be addressed (own observation).

Quality assurance is of utmost importance.

2.2 Accreditation: literature review

Although accreditation of medical laboratories was initiated in 1961, definite medical policies were not imposed globally and therefore the management of many medical laboratories still had the *choice* of being accredited or not in the 1990s! Several newsletters emphasized the necessity of accreditation and quality assurance, but sometimes to no avail.

Two distinct systems of total quality management applicable to medical laboratories exist: accreditation and certification. According to the ISO/IEC guide, by definition, 'accreditation' is a procedure by which an authoritative body gives formal recognition that a body or person is competent to carry out specific tasks. On the contrary, 'certification' is a procedure by which a third party gives written assurance that a product, process or service conforms to a specific requirement. In common usage to 'accredit' means to certify or guarantee someone or something as meeting required standards and to 'certify' means to endorse or guarantee that certain required standards have been met (Linko, 2003).

Globally, the organizations accrediting or certifying medical laboratories are of different types, i.e. governmental or authoritative organizations. The development of laboratory accreditation started, as it became clear to the United States Congress that unsatisfactory testing was performed within health care sectors. Consequently, the College of American Pathologists (CAP) initiated the first accreditation scheme in 1961 specially designed for medical laboratories: the Laboratory Accreditation Program (LAP). Today, the CAP program is recognized by the Joint Commission on Accreditation of Healthcare Organizations (JCAHO) and has a decision authority under the Clinical Laboratory Amendments of 1988 (Roberts, *et al*., 1987).

In Australia the National Association of Testing Authorities (NATA) as a principal inspection agency, has experience with accreditation for over 50 years mainly for the benefit of Australian industry, government and the community. A medical testing program
was established by NATA in 1983 to accredit pathology facilities. The Australian principles of accreditation follow the ISO 9000 standard series and ISO/IEC Guide 25. The Canadian Council on Health Service Accreditation (CCHSA) introduced a Client Centered Accreditation program in 1995 focusing on the implementation of Total Quality Management in medical laboratories.

To demonstrate the required procedures, European medical laboratories started to take action during the 1990s in developing their quality systems. The first medical laboratories were accredited in Sweden in 1992 by SWEDAC, the Swedish accreditation body (Linko, 2003). Since then, the number of accredited laboratories, representing disciplines of clinical chemistry, clinical microbiology, blood banking and pathology has been growing exponentially in the Nordic countries. Today, more than twenty medical laboratories in Finland have fulfilled the accreditation requirements assessed by FINAS, the Finnish Accreditation Service. In the United Kingdom, medical laboratories follow the national standards set by the Clinical Pathology Accreditation (CPA), which serves also as the national accreditation body (http://www.cpa-uk.co.uk).

In addition to analytical issues, guidance for documenting and implementing some special actions has been taken in account. These actions include, e.g. internal audits, an important management tool which medical laboratories might not have been so familiar with before (Linko, 2003).

The new international standard ISO/IEC 17025 replaced the criteria of the EN 45001 and ISO/IEC Guide 25 standards for laboratory accreditation by the end of 2002.

Mentioned below, are some medico-legal cases that were initiated in various countries where the laboratories concerned had to prove their quality management and assurance in courts of law:

Between 1993 and 1994 there were three incidents in Australia and the United Kingdom of laboratories causing a failure in a cervical screening program by under-reporting cervical cytology. The Cervical Screening Advisory Committee brought these screening failures to the attention of the National Coordinator of the Program and the Ministry of Health. Quality assurance policies had to be revised and implemented as a matter of urgency. (Literature does not confirm the dates of accreditation of these laboratories).

In June 1994, a hospital pathologist at Goodhealth Wanganui was found to have misread biopsy specimens. The pathologist was 62 years of age and had been diagnosed with Parkinson 's disease in late 1993. A review of his work revealed that he did not participate in quality assurance activities; he did not participate in continuing medical education and he was working in an isolated environment. It was recognized that all of these circumstances may have impaired his work as pathologist. The Cervical Screening Advisory Committee had to once again revise and implement various quality assurance requirements.

A newsletter of January/February 1993 contains an article on the accuracy of smear tests of 237 women referred to the Royal Hospital of Women in Sydney for invasive cervical cancer. The article noted that a worrying aspect was the number of patients whose previous smears on review, showed frankly malignant cells but were originally reported as normal.

In a second newsletter of March/April 1994, there is an article on a screening failure in Great Britain which described how a group of 2 000 women were recalled in Grennock where smears had been wrongly read for five years in a laboratory described as understaffed, antiquated and isolated. The Advisory Committee advised the national coordinator that appropriate quality assurance is mandatory.

During 1991, a Ministerial inquiry was initiated into the under-reporting of cervical smear abnormalities in the Gisborne region. It was found that the pathologist was the only primary cytology screener and that the laboratory only received approximately 4000–5000

cervical smear tests per annum. There was inadequate internal quality control as the pathologist did a 10% re-screen once a week and also had no other medical professional to consult with. The pathologist also did not compare the re-read results with the original results. There was also no correlation of biopsy results with cytology results and no external quality assurance program. The laboratory was not accredited with an independent quality control authority and the pathologist participated very rarely in continuing medical education. Even though the accreditation requirements for the Testing Laboratory Registration Council (TELARC) were not as demanding in the early 1990s as they are now, they still would have deterred this pathologist from practicing as he did. Most importantly, from 1993 onwards, as long as Gisborne Laboratories employed only one person to carry out all cervical cytology, it would have been denied accreditation (Ministerial inquiry, 1991).

Factors relating to the delivery of cytological services in New Zealand between 1990 and March 1996:

From the years 1990 to 1996 cytological services in New Zealand were delivered in circumstances where:

- Laboratories reading cervical cytology were not *required* to follow quality control processes or to be accredited with an independent quality control authority;

- *The Government Policy for National Cervical Screening* (1991) and the 1993 updated version in relation to laboratories reading cervical cytology were not well designed;

- The National Cervical Screening Register was not functioning optimally;

- There were no performance standards for laboratories, and there were no reliable data on laboratories' performance;

- There was no monitoring and evaluation of the performance of laboratories reading cervical cytology;

- The health authorities did not take heed of the warnings provided by the failures of cervical screening laboratories in other countries;

- There was a failure to ensure all components of the program were in place from an early stage.

- Compulsory accreditation for all cytology laboratories was not introduced until late 1996. It is difficult to be precise about when these requirements were introduced, as their introduction into individual laboratories was achieved at different times and through more than one mechanism. It is clear that before this time, accreditation and quality assurance was not mandatory, even though the need for quality assurance of cytology laboratories was seen as essential by more than one authoritative source from as early as the mid-eighties (Ministerial inquiry, 1991).

Similar situations occurred globally and accreditation and quality assurance became compulsory for all laboratories, within a certain time limit.

Accreditation authorities had to learn from the above experiences and had to implement quality assurance programs that ensure that each laboratory maintains the highest level of performance and quality assurance.

South Africa is now firmly part of the global market place and globalization increases the need for local accreditation and associated systems that are accepted internationally. Already many countries' national accreditation bodies, such as SANAS in South Africa, have come together to create an international platform. International Laboratory Accreditation Cooperation (ILAC) focuses on laboratories and inspection. It has over 60 members (http://www.sanas.co.za).

In November 2000, ILAC formalized an international Mutual Recognition Arrangement amongst 36 of its members who had been successfully evaluated for compliance with pre-agreed criteria for competent operation of laboratory accreditation programs. SANAS was among the founder members of this arrangement.

The International Accreditation Forum (IAF) focuses on certification and also inspection. It has 25 members and at the moment focuses primarily on quality management certification. As a member of IAF and ILAC, SANAS represents South Africa in all matters related to accreditation in conformity assessment (http://www.sanas.co.za).

The Ampath laboratory in Port Elizabeth was successfully accredited in November 2001**.** The South African National Accreditation Society (SANAS) accredits various laboratories with the view of accrediting all South-African laboratories within a certain time limit.

The accredited laboratories have to uphold a very high standard of quality to remain accredited. Teams of professionals inspect the laboratory every 2 years and other quality assurance staff inspects the laboratories every few months.

2.3 Accreditation procedure:

To have a laboratory accredited, entails a lot of hard work, preparing standard operating procedures and it is a very nerve-racking experience when the team of specialists from SANAS comes personally to the laboratory and inspects every department.

They do a very thorough check of various facets of the company, namely:

2.3.1 The organization and Management

1. The laboratory's practice number and registration number has to be displayed. No temporary or mobile facilities are allowed.
2. A detailed organogram as well as a clearly defined technical managerial hierarchical structure of the entire management has to be clearly displayed in the laboratory.

2.3.2 Quality management

1. Regional as well as departmental quality policies and manuals have to be readily available and updated at all times.

2. SHE policies and procedures. (These policies and procedures are reviewed annually and amended accordingly. Should law alter the policy for sick leave, the appropriate policy should be amended immediately).
3. All new methods and equipment evaluated and/or reviewed and documented accordingly. (Should the method of staining a slide be altered, the standard operating procedure should be changed accordingly).

2.3.3 Personnel

Each member of staff has his/her own personnel file with the following:

1. Curriculum vitae
2. Detailed job description
3. Current registration certificate with HPCSA (if necessary)
4. Health record
5. Detailed training record

6. Disciplinary actions and warnings
7. In-house work experience record
8. Attendance register of all meetings and seminars attended

2.3.4 Specimen collection

1. Various SOPs for specimen collection, storage, handling of specimens and safety precautions have to be in place and readily available in the laboratory
2. A SOP for labelling of specimens has to be in place.
3. A SOP describing the appropriate transport medium has to be available.
4. Preparation of the patient prior to specimen collection has to be documented on the specified form.
5. Documented instructions are needed for special storage arrangements of specimens.
6. Precautions should be taken with specimens that are damaged and unsuitable.
7. Safekeeping of all records is very important, as abnormal results should be kept forever and normal results for at least 10 years.

2.3.5 Specimen request form

The following details are required on this form:

1. Time and date of taking specimen
2. Name, address and/or location of hospital
3. Age and gender of patient
4. Name of the health professional requesting the test
5. Minimum relevant clinical data for the test
6. Date and time specimen arrives in laboratory

2.3.6 Specimen reception

SOPs are required for the following procedures:

1. Handling of specimens in reception area
2. Documented method of reception

3. Documented protective measures
4. Handling of urgent specimens
5. Handling of specimens after hours

2.3.7 Specimen identification

SOPs are required mentioning all the following procedures:

1. Unique identification number for each specimen
2. Identification number quoted on all documentation pertaining to this specimen
3. Laboratory policy for handling and/or rejection of unsuitable specimens or Inadequately labelled samples
4. Documented procedure for recording dispatch and receipt of referred specimens to and from laboratory

2.3.8 Test methods

SOPs are required with all the following details:

1. Validation of test methods recorded
2. Detailed step by step instructions for each test procedure
3. Principle of method
4. Storage and preparation of materials
5. Calculations and calibrations controlled and recorded
6. Identification of potentially hazardous operations or materials
7. Signature of person who checked and authorized method for use

2.3.9 Quality assurance programme

Each laboratory should have an updated copy of the following:

1. Written quality assurance programme / policy
2. In-house safety guidelines
3. Policy regarding hazards
4. Safety of staff and testing personnel

2.3.10 Test reports

Each test report should contain the following:

1. Patient's name, address and hospital number
2. Name and address of clinician requesting the test
3. Laboratory accession number
4. Date and time of collection
5. Name and location of laboratory performing the test
6. References to the tests requested
7. Clear and concise results
8. Date and time of issue of report
9. Signature and title of person taking responsibility for content of result.

2.3.11 Records

All records are checked to see whether the following rules are adhered to:

1. Identify person performing the work
2. Alterations signed and dated by authorized person
3. Documented procedures to minimize transcription errors
4. All records kept according to guidelines of Royal College of pathologists – Retention and storage of pathological records and archives
5. Records kept in suitable filing system
6. Patient data and reports only reported to the referring practitioner
7. Records of all equipment, e.g. Manufacturer, date received, current location, service planner, non-conformance list etc.

2.3.12 Safety and fire

The safety officer keeps strict control on the following:

1. Records and monthly feedback
2. Documented fire drills

2.3.13 Customer services

1. Customer services
2. Is a questionnaire available for doctor/client completion

3. Were pathologists and science/technical personnel available to the doctor/client

These are some of the facets of laboratory work that SANAS ensures are kept in place at all times by all Accredited laboratories!

2.4 Specific contributing factors affecting quality assurance in the diagnosis of cervical smears:

With the above as background, discussion will be directed to the following factors:

2.4.1 The taking of the smear:

As discussed before, the taking of the smears forms the most integral part of quality assurance. Formal training in smear taking is essential if the cervical smear test is to be reliable and such training is increasingly available. With the patient in the lithotomy position, the clinician should visually inspect the cervix and identify the transformation zone.

Then, if possible, sampling should be directed to encompass the entire area, including both the endocervix and ectocervix.

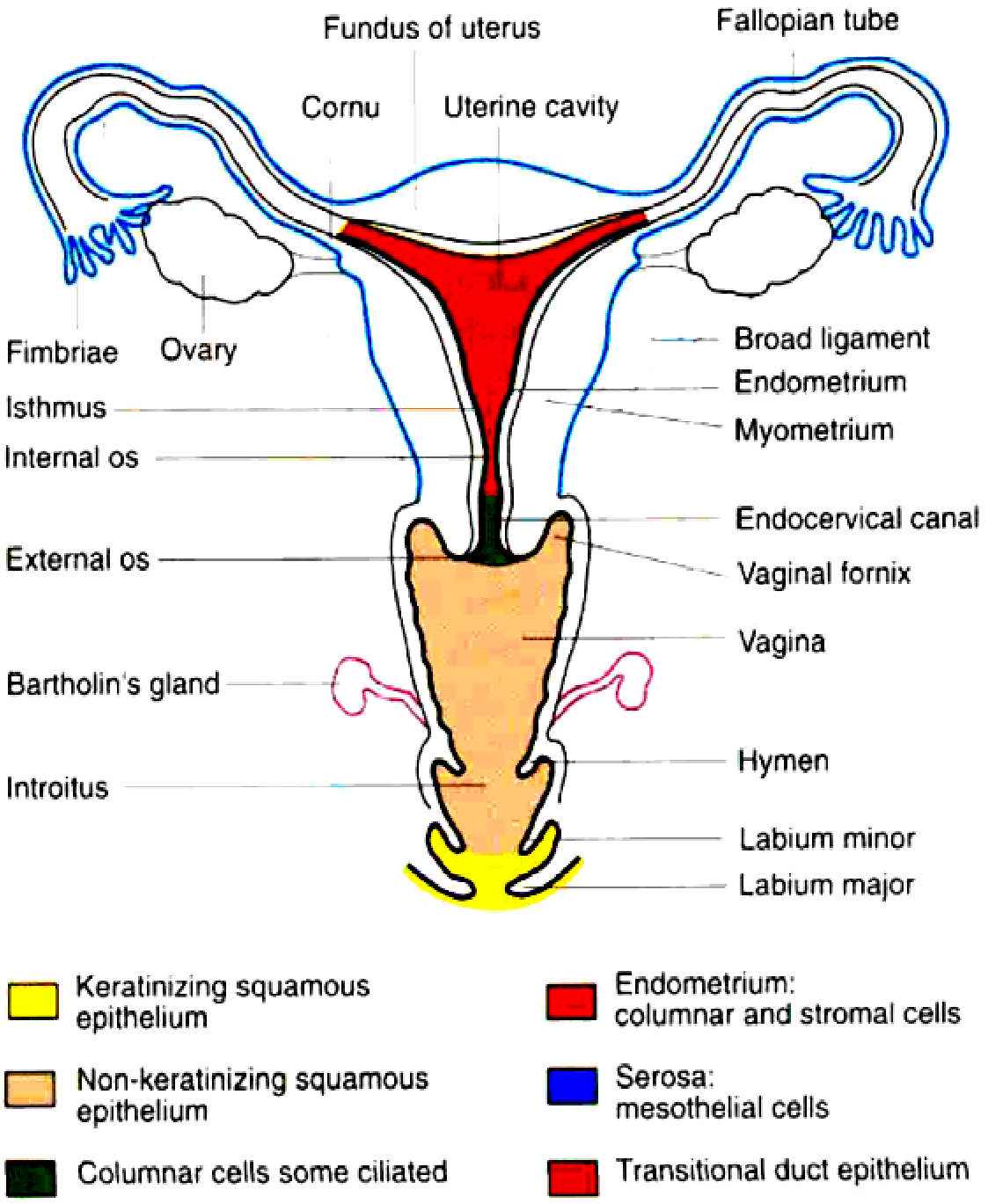


Diagram 1: Anatomy of female genital tract (Taken from Diagnostic pathology)

The point at which the ectocervical squamous mucosa meets columnar cell mucinous epithelium of the endocervix is the squamocolumnar junction (Diag. 1). This point is not static and moves proximally through the transformation zone as inflammation, infection, or other irritations necessitate that the distal canal be lined by a heartier squamous

epithelium. It can then recede when conditions normalize (Fig. 9). Thus, the concept of a zone rather than a junction seems clear (Geisinger, 2003).

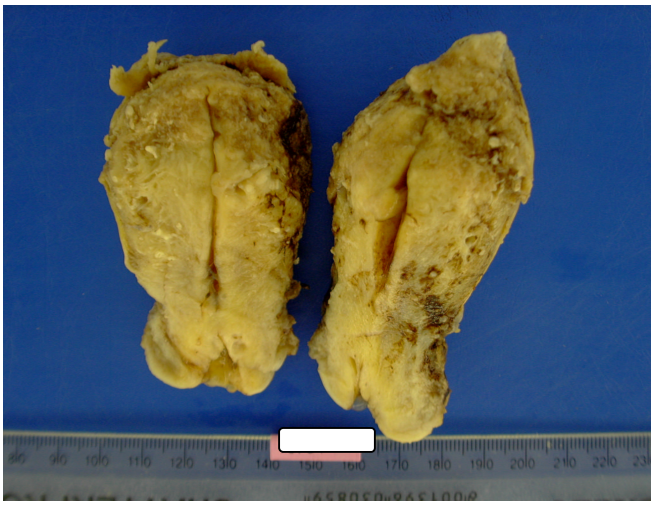


Figure 9: Example of an opened cervix and uterus
(Data taken from Ampath laboratory Port Elizabeth)

As a quality measure, the proportion of inadequate or unsatisfactory smears in relation to the entire smear workload of a laboratory provides a valuable indication of the standard of reporting and of the level of expertise of the smear takers (Valenzuela, *et al*., 2001).

2.4.1.1 Criteria for assessing smear quality:

The question of adequacy of cervical smears is central to the success of cervical screening in the prevention of cervical cancer deaths. The problem of establishing exactly what constitutes an adequate sample has received increasing attention in recent years, culminating in broad guidelines from various sources. According to the Bethesda system, 8000 to 12000 well-preserved and well-visualized squamous cells plus 2 groups of at least 5 endocervical cells or squamous metaplastic cells is acceptable as adequate. Because the primary purpose for a cervical test is identification of squamous lesions, this is directed to these cells. It is important to note that cell-by-cell counting has not been recommended, indicating that the cell number is to be estimated (Geisinger, 2003).

The method for estimating cellularity has not been fully developed. Some inherent smear characteristics might be expected to contribute to variations in apparent cell numbers, e.g. cytolysis, relative cell crowding, atrophic smears, mucus, inflammatory cells. Given these confounding visual factors, the difficulty and subjectiveness involved in cell number estimation, becomes clear. The proposed solution is comparison of the slide in question with reference images derived from smears showing various well-quantified levels of squamous cellularity.

It seems logical to expect that, when looking at a number of fields on a conventional smear with questionable cellularity, these fields are likely to differ considerably from one another. Thus the cytologist may be in the position of testing several fields, perhaps against a number of different reference images and then mentally calculating a quasi-arithmetic sum of several subjective assessments. The reproducibility of this method remains to be investigated (Geisinger, 2003).

Atrophic smears (fig. 10) differ from the smears of younger women and therefore it is important for the screener to know the patient's age and menstrual status and of any hormonal treatment. (Atrophic smears show mainly parabasal cells and some intermediate cells due to low estrogen and the smear from a younger woman shows mainly superficial cells and intermediate cells due to higher estrogen effect).

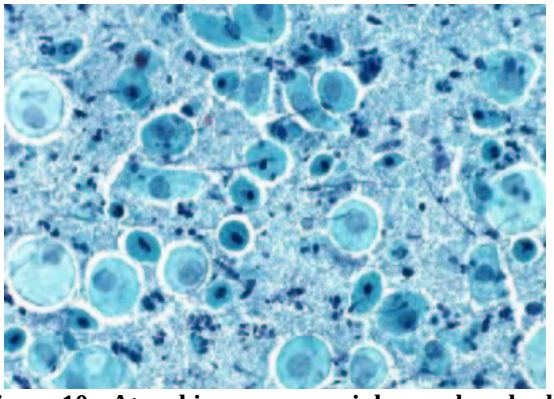


Figure 10: Atrophic smear – mainly parabasal cells

(Taken from The Bethesda System for reporting cervical cytology)

As a working principle, cervical smears should consist of clearly displayed cellular material covering at least one third of the area under the coverslip, and preferably over one half. The medical professionals combine ecto- and endocervical smears on one slide to save the patient the extra expense. The mere inclusion of columnar or metaplastic cells, or both, does not necessarily confirm that the entire circumference of the transformation zone has been sampled. The clinician taking the smear has to be relied upon for confirming the thoroughness of the sampling procedure. Squamous cells of cervical origin are usually distributed in loosely cohesive streaks along the lines of spread of the smear.

Whether smears without any endocervical cells or recognizable metaplastic cells should be accepted as representative, is debatable. The presence of these cells is determined largely by the position of the squamocolumnar junction and the state of maturation of the transformation zone, factors that are hormone dependent. Thus it may not always be possible to include endocervical cells or identify metaplastic cells at all times. An additional factor is the nature of the sampling device, the Aylesbury spatula giving a greater yield from within the canal than the Ayre spatula. Brushes and broom devices provide the largest endocervical component. There are many reports of comparative trials of the different smear taking instruments, the general conclusion being that devices with an extended arm give a more representative sample and have a greater likelihood of

including abnormalities(Fig.11).

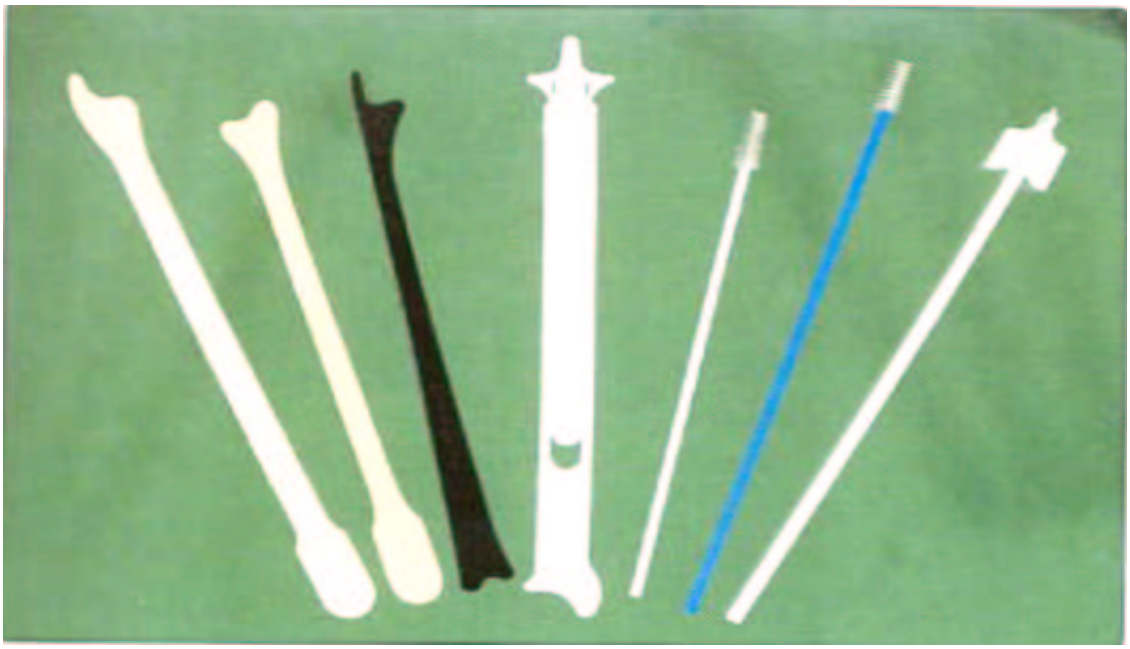


Figure 11: Various spatulas used for taking cervical smears

(The Ayre spatula second from left, the Aylesbury spatula far left and brushes and broom-like devices far right.) (Taken from Gray and McKee, 2003).

In a study of 635 patients with previous abnormal cytology referred to colposcopy, Henry and Solomon (1995) examined the effect of the amount of endocervical component on the detection of squamous lesions.

The presence of one to five endocervical cell clusters increased the number of HSIL cases detected from 6% to 10%. The presence of greater numbers of endocervical cells had no additional effect on the detection rate of HSIL. Interestingly, the presence of endocervical cells did not affect the detection rate of LSIL (Low Grade squamous intraepithelial lesions). In fact, large numbers of endocervical cells actually decreased the rate of detection of LSIL from 21% when one to five clusters were present, to 10% when greater than 25 cell clusters per slide were present. This may be a reflection of sampling too far up into the endocervix, thus missing lower grade lesions which are more commonly found on the ectocervix (Apgar,*et al.*, 2003).

An excess of leucocytes obscuring epithelial cells (Fig. 12) may require investigation for a treatable cause, especially if a discharge is present. Similarly, unsatisfactory smears

taken postnatally should be repeated when normal menstrual cycles are re-established (Gray and McKee, 2003).

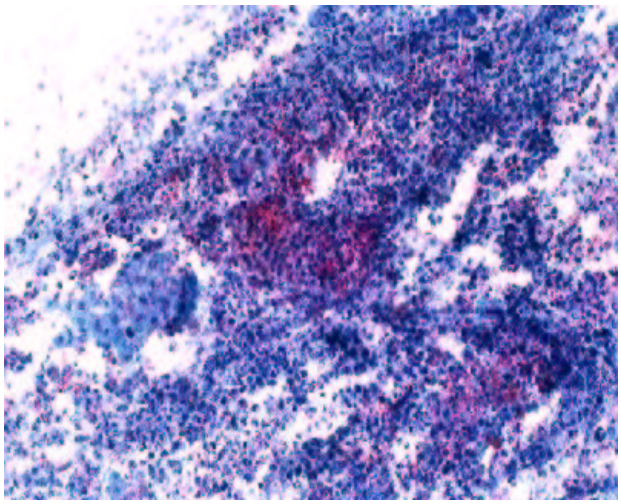


Figure 12: An example of an unsatisfactory cervical smear

(Note - no *well-visualized* cells could be identified due to obscuring white blood cells).

(Taken from The Bethesda system for Reporting cervical cytology).

Blood–stained smears are often poorly fixed or obscured by the blood (Fig. 13). Contact bleeding on smear taking may be due to an ectropion, requiring treatment before a satisfactory smear can be obtained. The epithelial cells are sometimes so obscured, that a definite diagnosis is not possible (Apgar, *et al*., 2003).

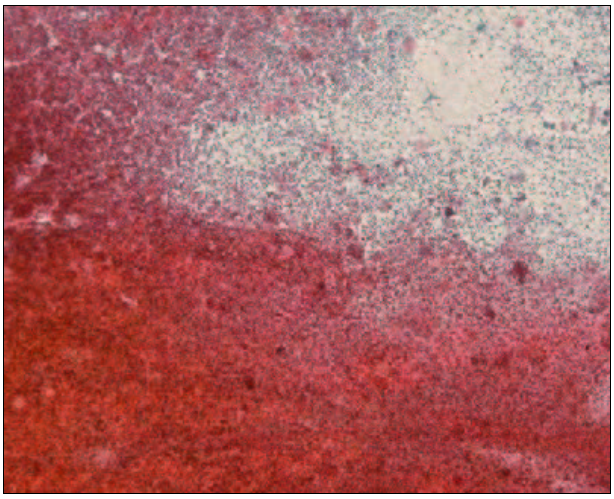


Figure 13: An example of a cervical smear obscured by red blood cells.

(Note - If 50% - 75% of the epithelial cells are covered, obscuring red blood cells should be mentioned in the quality indicators of the report).

(Taken from The Bethesda system for Reporting cervical cytology).

Post-menopausal atrophy leads to scanty or inflamed smears, often with no endocervical cells. The threshold of acceptance of smears from these women must be adjusted, but if there is cause to doubt the adequacy of sampling, a short course of local estrogen cream prior to smear taking usually ensures better sampling (Gray and McKee, 2003).

2.4.2 Fixation of smear:

Rapid fixation is essential to ensure the cells are well preserved. Fixatives are applied to a smear or by immersion of the slide into a liquid. The liquid fixatives include 95% alcohol, polyethylene glycol and/or carbowax. The 95% alcohol is inexpensive, readily available and provides excellent cytological detail. Its only disadvantages are the need to store and transport unstained slides in a container of liquid and to have the fluid and the containers at hand at the clinics or doctor's rooms (SOP-LP-PCYTO-013).

The alternative to wet fixation is the use of commercially available spray fixatives. These generally consist of various mixtures containing ethanol or isopropyl alcohol with carbowax (polyethylene glycol). The latter leaves a coating on the slide after the alcohol has evaporated and is said to prevent cell shrinkage.

Such sprayed slides are dry within a few seconds and are then suitable for storage, mailing, or immediate processing. These coating fixatives must first be removed with a soak in 95% alcohol before staining is performed. If this step is hastened, poor staining quality results.

Aerosols expand isentropically when sprayed and thus are very cold. If the spray is held closer to the smear surface rather than about 25cm from it, the cells become rapidly frozen and severely damaged. The microscopic result often renders the material unsuitable for cytological examination (Geisinger, 2003).

Regardless of the fixative selected, it must be applied quickly because good results are obtained only if it is applied within a few seconds of making a smear. Severe drying effect renders cervical smears unsuitable for interpretation (Geisinger, 2003).

2.4.3 Marking of the slide:

The slide should immediately be marked on the frosted end of the slide with a pencil or diamond pen. The patient's surname or the exact site of the smear taken should be written here thus preventing slides being mixed up with other patient's forms, resulting in the incorrect slide being screened with the incorrect form. When the clinician decides to take two smears, the slides should also be marked accordingly.

Most clinicians do not follow this rule, but wrap the slide in its corresponding request form and put an elastic band around it. The laboratory assistant then opens these slides one by one and marks the slides personally according to the name on the appropriate form wrapped around it (SOP-WI-PCYTO-001).

2.4.4 Submission of clinical data:

Laboratory test results are of limited use in the absence of corresponding clinical information. The range of necessary and pertinent information is broad but may range from only the patient's age to complete information inclusive of the clinical history and physical examination for proper interpretation of a test result (Fig. 14).

Clinical information is critical for the technologists, pathologists and clinicians alike with the ultimate goal of optimal patient care (Marques and Mc Donald, 2000).

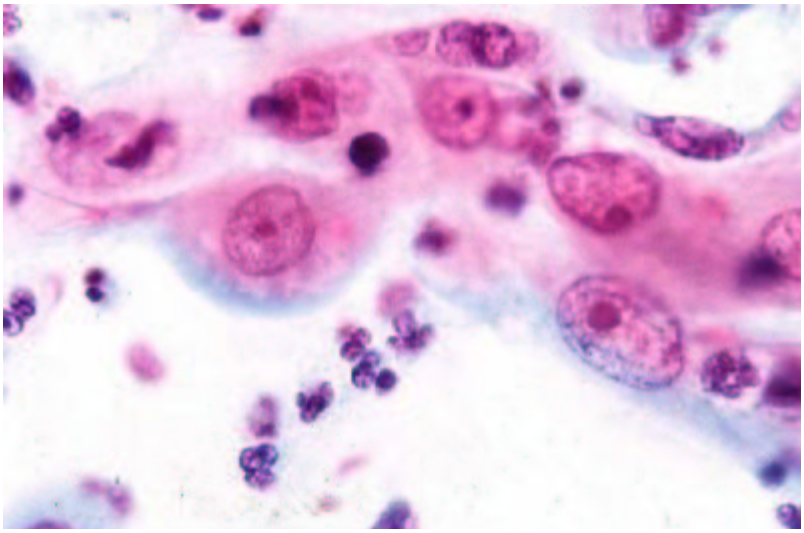


Figure 14: Reactive changes – no clinical data

(Could have been mistaken for abnormal cells)

(Taken from the Bethesda system for reporting cervical cytology)

2.4.5 Transportation of cervical smears:

Timeous Cytoprep-fixation is the perfect "transport medium" for a cervical smear and this fixation will keep the cells in perfect condition for a couple of days. No ice packs or additional transport medium is needed (SOP-LP-PCYTO-013).

2.4.6 Receiving of smear in the laboratory:

Training on the receipt of specimens in the laboratory is regarded as of utmost importance as NO mistakes may be allowed here. Only one specimen is opened at a time with its corresponding form. The information on the slide is compared with those on the form, and if correct, the slide is assumed to belong to that form and both the slide and the form are allocated a unique laboratory number. This number is also entered into the daybook and all relevant data written next to this number.

If the slide is unmarked but individually wrapped in its particular requisition form, the name of the patient and the laboratory number is written on the frosted end of the slide

and a note is written on the accompanying form that there was no name written on the slide.

The particular sender is phoned immediately should there be any discrepancy as to whether the slide belongs to the form, or if the names differ. Unless the clinician's rooms can rectify this problem telephonically, the smear and form is returned to the rooms to be rectified (SOP-WI-PCYTO-001).

2.4.7 Staining of slides:

Laboratories vary in their way of staining cervical smears as some laboratories have automated staining machines with different pre-set staining times (Table 1). Other laboratories stain manually, but in both cases the normal Haematoxylin – Eosin Pap stain is usually used.

Quality control of the stained slides is of utmost importance and the quality of the staining of every slide is documented on every individual worksheet. Corrective action is taken accordingly. (Should there be a batch of a certain stain or other chemical that is faulty, it should be replaced before the next slides are stained).

The filtering of the contents of the staining dishes is also very important as this avoids contamination from one batch of smears to the next (SOP-WI-PCYTO-008).

The slides are first placed in a dish with 95% alcohol to dissolve the carbowax of the fixative used by the clinician. As Haematoxylin is a water based stain, the cells would fall off the slides if they were directly placed in this stain.

Placement in 80% alcohol, 70% alcohol, 50% alcohol and then water hydrates the smears. The cells are now hydrated and can be placed in the Haematoxylin stain. Haematoxylin stains mainly the nuclei of the cells. Scott's solution is used as blueing

agent and then the smears are dehydrated again as the OG6 and EA65 stains are alcohol based and stain the cytoplasm of the cells.

The dishes with the slide racks are thus placed into 50% alcohol, 70% alcohol and then 80% alcohol to eventually get to 95% alcohol (methanol).

The cytoplasm of the cells are stained with the OG6 and EA65 stains and washed in methanol.

Before the slides can be placed in xylene – a clearing agent, a mixture of 50% methanol and 50% xylene is used, as xylene is oil based.

The slide rack is then placed in the xylene for +/- 5 minutes to clear.

The slides are not to be removed from the xylene prior to mounting (SOP-WI-PCYTO-008).

Table 1: Papanicolaou staining method (Taken from SOP-WI-PCYTO-008)

PROCEDURE:

1	95% Alcohol	5 minutes	(fix smear and dissolve carbowax)
2	70% Alcohol	10 seconds	(hydrate smear)
3	50% Alcohol	10 seconds	
4	Water	20 seconds	
5	Haematoxylin	6 minutes	(water base – stain nuclei of cells)
6	Water	10 seconds	
7	Scott's	2 minutes	(blueing agent)
8	Water	10 seconds	
9	Water	10 seconds	
10	50% Alcohol	20 seconds	(dehydrate smear)
11	70% Alcohol	20 seconds	
12	80% Alcohol	20 seconds	
13	Methanol	20 seconds	(95% alcohol)
14	OG6	2 minutes	(alcohol-based dye – stains cytoplasm)
15	Methanol	20 seconds	
16	Methanol	20 seconds	
17	EA65	2 minutes	(alcohol-based dye – stains cytoplasm)
18	Methanol	20 seconds	
19	Methanol	20 seconds	
20	Methanol	20 seconds	
21	Methanol / Xylene	20 seconds	
22	Xylene	20 seconds	(clearing agent)
23	Xylene	20 seconds	
24	Xylene	5 minutes	

The smears are now ready to be mounted with appropriate mounting medium.

2.4.8 Mounting of slides:

Since xylene is a known carcinogen, all mounting of slides should be done under an extractor fan. Entellan of DePex can be used as a mounting medium. Cross-contamination (cells from the previous staining batch picked up by the slides of the next

batch) and air-bubbles are avoided at all times since an air bubble can obscure abnormal cells (Fig. 15). Cross contamination is avoided by filtering the contents of all the staining dishes.

As the cervical smears have different areas of smear thickness, the mounting medium sometimes do not cover the thick areas and therefore form air-bubbles. The slide is then placed back into a jar with xylene until the coverslip loosens and the slide can be remounted. Then only can the slide be screened.

The quality of the mounting of every slide is documented on every individual worksheet and corrective action taken if necessary (SOP-WI-PCYTO-010).

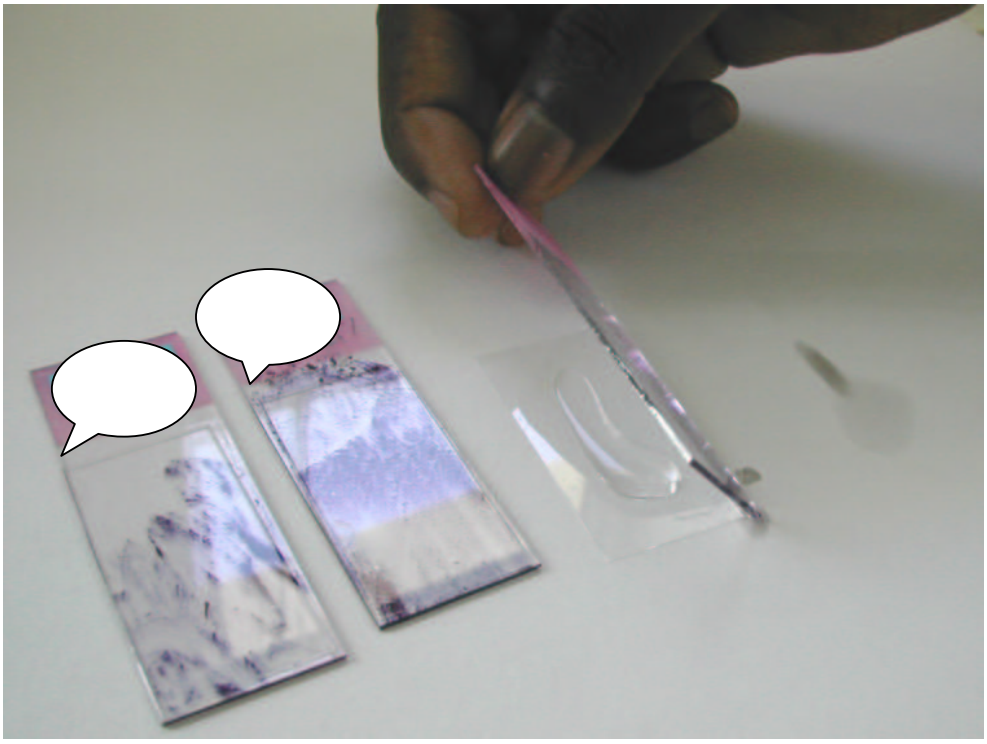


Figure 15: Mounting of slides
(Taken from Ampath laboratory Port Elizabeth)

By law, all abnormal smears must be retained indefinitely – therefore the quality of the mounting medium must also be assessed, since the quality of the staining must be

maintained for future referral to the slide. If need be, the slide can be placed in xylene to remove the coverslip and then the smear can be restained as required.

The size of the coverslips used is a very important component of quality assurance. Pathology laboratories differ in opinion on the percentage of abnormal cells missed when using smaller coverslips (Oommen, *et al.*, 1998). There is a very big variety of coverslips – varying sizes and some are round, others are made of glass and others of plastic.

The patient is entitled to an as thorough as possible evaluation of the smear evaluated and therefore the author's laboratory agrees on using the larger coverslip (24mm x 50mm).

2.4.9 Labelling:

Before a slide is labelled, the name and number on the slide as well as the form is compared and the appropriate label is attached to the frosted end of the slide. This is the third quality assurance check ensuring the slide has the correct name, number, label and accompanying form (SOP-WI-PCYTO-013).

2.4.10 Screening:

2.4.10.1 Manual screening:

When properly obtained and prepared, cervical smears alert practitioners to possible abnormalities of the cervix.

Manual microscopic screening of cervical smears by cytotechnologists continues to be the most widely used and accepted method of identifying irregular cells on cellular abnormalities of the cervix (Cronje, 2004).

During manual screening of the cervical smear samples, cytotechnologists search for what may be a few abnormal cells by inspecting many fields on a slide under the microscope.
Cytotechnologists must manipulate the microscope stage through visual inspection of approximately 50 000 to 300 000 epithelial and inflammatory cells per smear (De Palo, 2004).

Manual screening can be tedious and time-consuming since the majority of cells on the smear are normal. Even smears with serious abnormalities may contain only a few dozen malignant or premalignant cells among hundreds of thousands of normal cells.

The screener checks on the previous records of every patient and any previous results or slides are retrieved for reviewing. These findings are then compared to the current slide on this patient (SOP-WI-PCYTO-032).

Any cell that is worrisome to the screener is marked with a permanent marker and marked as such at the bottom of the worksheet. The presence of transformation zone cells is also marked on the coverslip with a "T". The slide and worksheet is then handed to the senior checker.

This slide is rescreened and discussed with the particular screener. An opinion is formulated and the slide then may still be shown to the pathologist in charge (Freckelton, 2003).

False-negative cervical smears:
A false-negative cervical smear is defined as a normal cytological report of a woman with existing dysplasia, premalignant or malignant lesions of the cervix. When these cells are missed, it results in delayed diagnosis that can lead to progression of the disease and the need for more aggressive treatment (Geisinger, 2003).

False negatives can occur under the following conditions:

Cell sampling and preparation:

Accuracy of cervical smear results depends in part upon how well the cervical material is sampled and slides prepared. At the time of collection, samples of cells collected from the surface of the cervix must contain both ectocervical and endocervical cells. If abnormal cells are not included in the sample, abnormalities cannot be detected.

False negative results can also occur if blood, vaginal secretions, or inflammatory cells in the sample conceal the abnormal cells making them undetectable by the human eye.

Screening and interpretation:

False-negative results, due to screening, are caused by failure to identify a relatively small number of abnormal cells in the screening process. A trained cytotechnologist typically searches hundreds of thousands of cells per smear on up to 70 slides per day (SAMHSA TIPs 2004).

Factors that may make a smear more likely to receive a false-negative interpretation:

Few abnormal cells present

Abnormal cells present on only part of a conventional smear

Abnormal cells present singly rather than in groups

HSIL cells that is small

HSIL cells that lack nuclear hyperchromasia

QUALITY ASSURANCE IS THEREFORE THE KEYWORD FOR EVERY CYTOLOGY LABORATORY.

With hundreds of thousands of cells on every slide, the screener depends heavily on quality
assurance steps 1 to 9, as this will make his/her job much easier.

Liquid-based preparations:

There is a worldwide shortage of trained cytotechnologists and cytopathologists and the situation in the UK has currently reached crisis point. There is much anecdotal evidence that liquid-based preparations are very popular with cytotechnologists.

Liquid based cytology, or thin layer cytology, is a technique of preparing a monolayer of cells on a glass slide. The technique involves suspending the sample in a solution that is both mucolytic and haemolytic. The sample is concentrated either by centrifugation or by filtration. In the former method a monolayer of cells sediments onto the slide, in the filtration method a monolayer of cells is pressed onto the slide from the filter.

The clarity of the presentation, the improved cell preservation and lack of overlapping by other cells, blood and inflammatory exudates are much appreciated. Depositing a representative sample on a discreet small area of the glass slide allows faster and more reliable assessment by laboratory staff. The cells are in a predetermined smaller area on the slide and are mainly in one focus plane when using the x 10 screening objective.

However, before a laboratory converts a cervical cytology service to liquid-based cytology, serious consideration must be given to some important issues. Smear takers must use a broom-style plastic sample collection device.

Smear taker training in the new technology is critical in that the sampling device must be rotated five times around the full circumference of the transformation zone to collect enough material for an adequate sample. Care must be taken to transfer all the cellular material on the head of the broom or the head of the broom itself if using the AutoCyte-Prep, into the vial immediately (Gray and McKee, 2003).

Material management should also be seriously considered. The delivery of supplies of preservative fluid to the health professionals and clinics and storage space within those clinics should be identified. Vials of preservative fluid have a long shelf life but storage and stock control of collection fluid is required. The transport of cell suspensions rather than dry glass slides to the laboratory may need different arrangements. The vials are bulkier, and thus require greater capacity in the collection vehicles (Gray and McKee, 2003).

In the laboratory, an additional resource is required to produce the new slide preparations. Conventional cervical smears arrive in the laboratory already "prepared" and only require to be stained and coverslipped.

Liquid-based cytology samples require preparation first and this has equipment, consumable and staffing resources attached to it. In addition, since the microscopic appearance of liquid-based preparations differ from conventional smears, staff must learn how to interpret these new appearances as well as recognize new 'alarms' to replace the loss of the more familiar clues in conventional smears.

Advantages of liquid based cytology

- Clean background
- Excellent fixation
- Well defined nuclear detail
- Instant preservation with no air drying artefact
- Removal of blood and mucus
- Smaller screening area 19-12mm^2 containing 50,000 - 70,000 cells
- Fewer fields to screen
- Increased productivity
- Standardization of collection and laboratory techniques
- Reduction in unsatisfactory rates
- Reduction in low-grade abnormality rate

- Ability to use sample for other techniques such as HPV typing, immunocytochemistry or DNA probes.
- Ability to triage women with high-grade abnormalities through adjunctive diagnostic tests
- Reduction in costs of women unnecessarily referred for colposcopic assessment and treatment.

Disadvantages of liquid based cytology

- Training needed for smear takers
- Lengthy laboratory preparation time
- Training required to operate equipment
- Training required to interpret the smears
- Morphological appearance may be different from conventional preparations
- Cost of reagents
- Storage of reagents and vials
- Loss of existing screener skills in interpreting conventional smears
- Loss of ability for future staff to develop skills in conventional smear interpretation

There is a steep learning curve and both cytotechnologists and cytopathologists must undertake significant additional training in order to achieve the same sensitivity and specificity on liquid-based preparations as for conventional smears.

Cost is a major consideration and the additional cost for consumables for laboratories is perceived as the disadvantage of liquid-based cytology (Gray and McKee, 2003).

2.4.10.2 Automation in cytology

Various attempts have been made to automate the microscopic component of cervical screening, which constitutes the main element of data gathering. The microscopic analysis of smears is not the only area where automation can have an impact. Important advances have already been made in the fields of sample preparation, data storage and manipulation.

Currently, machines are used to assist in staining and mounting slides, and computers are used to record details on patients with their results. The use of computerized data storage has assisted in the analysis of information and provided support to cervical screening programs in the production of printed results, letters and reminders to patients.

Electronic data transmission permits the rapid availability of results to clinics, surgeries and other destinations where cervical screening information is required for the management of the service (http://www.babst.com).

Over recent years automated devices to assist in the actual process of cervical screening have included:

The Pathfinder system developed by the Compucyte Corporation augments the manual process by providing assistance and feedback to the screener to measure the accuracy of the physical process of screening. The effect of feedback and process standardization has been
shown to decrease the number of missed minor abnormalities. Pathfinder may be a useful tool in the fields of both education and diagnosis by providing innovative management tools that ensure consistent high quality screening.

AccuMed International Inc. developed the AcCell Series 2000 automated slide handling and data management system that supports much of the screening process including referral from the clinic, log in, work collection, primary screening, review and rescreen, quality
control, and specimen archiving by image capture. The automated slide delivery is linked to an automated microscope and computer that enables human screening to take place.

Cytosavant is a fully automated high-resolution image cytometer developed by Oncometrics Imaging Corporation and Xillix Technologies Corporation. The principle

behind the system is to detect nuclear features that enable an objective diagnosis to be made. A camera captures an image at the highest spatial and photometric resolution that ensures the nucleus is in exact focus and precisely segmented. 150 nuclear features are calculated and a combination of decision tree processing and discriminate function analysis is used to classify cells and reject artefacts. The manufacturers aim to develop a procedure that eliminates 50% of samples with no squamous intra-epithelial lesions and to give positive samples a quantitative score that will triage patients for further follow up (http://www.aetna.com).

The Autocyte of Roche Inc. consists of a highly automated microscope and slide handler coupled to a computer. Images are captured and displayed at high resolution for a cytologist to interpret. The manufacturers believe that the dual strategy of human expert opinion with consistent unbiased computer decisions should generate a finer net to catch otherwise unrecognised abnormalities.

Neuropath's AutoPap 300 automates screening of conventionally prepared smears, it is a quality control device that reviews all smears classed as within normal limits and that are satisfactory, thus it helps to identify false negative smears and unsatisfactory slides. The machine consists of a high-speed video microscope and a field of view computer that interprets the image to provide a final classification based on object identification, object classification and slide classification. The system is designed to reduce the number of slides requiring human review, allow a laboratory to increase the number of slides processed, and decrease the costs per slide. Trials have shown that 60% of abnormal cells are identified.

AutoPap assisted screening has shown superior sensitivity and specificity when compared to current practice but meticulous attention to the preanalytical stages of staining and presentation is required for enhanced performance. The clinical use of AutoPap as a primary screening device should improve the overall practice of cervical cytology as well

as providing potential enhancement in laboratory productivity, however, the AutoPap is unable to classify atrophic changes and other benign cellular changes as normal.

Neuromedical Systems Inc. developed PAPNET, which uses a two-stage process involving the scanning and review of slides. Algorithmic processing is used to identify a set of
potentially abnormal objects followed by neural network processing to identify abnormal cells. The system selects 128 scenes with the highest neural network scores and electronically records the image for review by cytologists. Each case is triaged as either negative or requiring review with the latter being examined under a microscope. PAPNET has been shown to have a mean sensitivity of 97% for detecting significant abnormalities (http://www.aetna.com).

When used with conventional microscopy, PAPNET may provide the means to improve the accuracy of cervical cytology as it has been shown to be a good tool in detecting false negative smears.

When using PAPNET to rescreen smears, cytologists have found a significantly higher proportion of previously undetected cervical abnormalities as compared with conventional rescreening. Effectiveness estimates of interactive neural network assisted screening suggested that the sensitivity for detecting cervical epithelial abnormalities exceeded that of unassisted screening whether used as a substitute or an augmentation as PAPNET in tandem with rapid rescreening reduced the rate of false negatives in diagnostically difficult smears.

In 1997 International Academy of Cytology task force group examined computerized screening devices and made a number of recommendations that encompassed previous guidelines set out in 1984 that demanded 100% specificity and sensitivity. The professional in charge of a clinical laboratory should continue to bear medical responsibility for diagnostic decisions made by machines. Automation should not lead to
a reduction in neither standards nor expose the patient to any increased risk.

Supervisory personnel should continue a visual quality control on a percentage of slides diagnosed as normal, and recognition should be made that human expert opinion represents the gold standard.

The setting of performance criteria should remain the responsibility of the regulatory authorities, and not the manufacturers, and the professional cytology community should participate in the development of procedures that improve decision-making (http://www.cytology-iac.org).

Every cell is evaluated and screened very cautiously and reported on in a specific way acceptable and understandable by everybody concerned **(**SOP-WI-PCYTO-032). At this stage, the Bethesda 2001 is in use. This system has preferred nomenclature and recommendations (Davey, 2003)**.**

2.4.10.3 The Bethesda System:

The terminology for cervical carcinoma precursor lesions has changed several times during the years.

The traditional nomenclature, dysplasia and carcinoma-*in-situ* (CIS), has the advantage of being readily understood by many practitioners and being descriptive of the lesions (De May, 1999).

Cervical intra-epithelial neoplasia (CIN) was introduced to emphasize the spectrum of abnormalities that these lesions represent and to help standardize treatment.

The latest entry, the Bethesda System, divides precursor lesions into two groups: LSIL and HSIL, respectively. Equivalent terms are mild dysplasia, CIN I, and LSIL; and moderate dysplasia, CIN 2, and HSIL; severe dysplasia, CIN 3, and HSIL; and

carcinoma-*in-situ*, CIN 3 and HSIL. Human papilloma virus (HPV) changes and condyloma are considered LSIL (Apgar, *et al.*, 2003).

Table 2: The Bethesda system (Apgar, *et al.*, 2003)

BETHESDA SYSTEM 2001

SPECIMEN TYPE: *Indicate conventional smear (Pap smear) vs. liquid-based vs. other*

SPECIMEN ADEQUACY

_ Satisfactory for evaluation (*describe presence or absence of endocervical/transformation zone component and any other quality indicators, e.g., partially obscuring blood, inflammation, etc*)
_ 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*)
_ Negative for Intraepithelial Lesion or Malignancy
_ Epithelial Cell Abnormality: See Interpretation/Result (*specify* 'squamous' *or* '*glandular' as appropriate*)
_ Other: See Interpretation/Result (*e.g. endometrial cells in a woman > 40 years of age)*

AUTOMATED REVIEW
If case examined by automated device, specify device and result.

ANCILLARY TESTING

Provide a brief description of the test methods and report the result so that the clinician easily understands .

INTERPRETATION/RESULT

NEGATIVE FOR INTRAEPITHELIAL LESION OR MALIGNANCY (*when there is no cellular evidence of neoplasia, state this in the General Categorization above and/or in the Interpretation/Result section of the report, whether or not there are organisms or other non-neoplastic findings*)

ORGANISMS:
-Trichomonas vaginalis
-Fungal organisms morphologically consistent with *Candida* spp
-Shift in flora suggestive of bacterial vaginosis
-Bacteria morphologically consistent with *Actinomyces* spp.
-Cellular changes consistent with Herpes simplex virus

OTHER NON-NEOPLASTIC FINDINGS (*Optional to report; list not inclusive)*:
-Reactive cellular changes associated with
-inflammation (includes typical repair)
-radiation
-intrauterine contraceptive device (IUD)
-Glandular cells status post hysterectomy
-Atrophy

OTHER
-Endometrial cells (*in a woman > 40 years of age*)
(*Specify if 'negative for squamous intraepithelial lesion')*

EPITHELIAL CELL ABNORMALITIES

SQUAMOUS CELL

-Atypical squamous cells
- Of undetermined significance (ASC-US)
- Cannot exclude HSIL (ASC-H)

-Low-grade squamous intraepithelial lesion (LSIL)
Encompassing: HPV/mild dysphasia/CIN 1

-High grade squamous intraepithelial lesion (HSIL)
encompassing: moderate and severe dysplasia, CIS/CIN 2 and CIN 3

- with features suspicious for invasion (*if invasion is suspected*)

-Squamous cell carcinoma

GLANDULAR CELL

-Atypical

- endocervical cells (NOS *or specify in comments*)
- endometrial cells (NOS *or specify in comments*)
- glandular cells (NOS *or specify in comments*)

- Atypical
- endocervical cells, favor neoplastic
- glandular cells, favor neoplastic
- Endocervical adenocarcinoma *in situ*

- Adenocarcinoma
- endocervical
- endometrial
- extrauterine
- Not otherwise specified (NOS)

OTHER MALIGNANT NEOPLASMS: (*specify*)

EDUCATIONAL NOTES AND SUGGESTIONS (*optional*)
Suggestions should be concise and consistent with clinical follow-up guidelines published by professional organizations (references to relevant publications may be included).

The Bethesda System (Table 2)

Type of smear:

The conventional smear (Pap smear) is normally used in South Africa.

Specimen adequacy:

For **"**Satisfactory" specimens, information on transformation zone sampling and other adequacy qualifiers is also included. Providing clinicians/specimen takers with regular feedback on specimen quality promotes heightened attention to specimen collection and consideration of improved devices and technologies. Any specimen with abnormal cells is by definition satisfactory for evaluation. If there is concern that the specimen is

compromised, a note may be appended indicating that a more severe abnormality cannot be excluded. At least 8 000 – 12 000 well preserved and well-visualized squamous epithelial cells should be present. Ten well preserved endocervical cells or squamous metaplastic cells, singly or in clusters renders an adequate transformation zone component (Fig. 16).

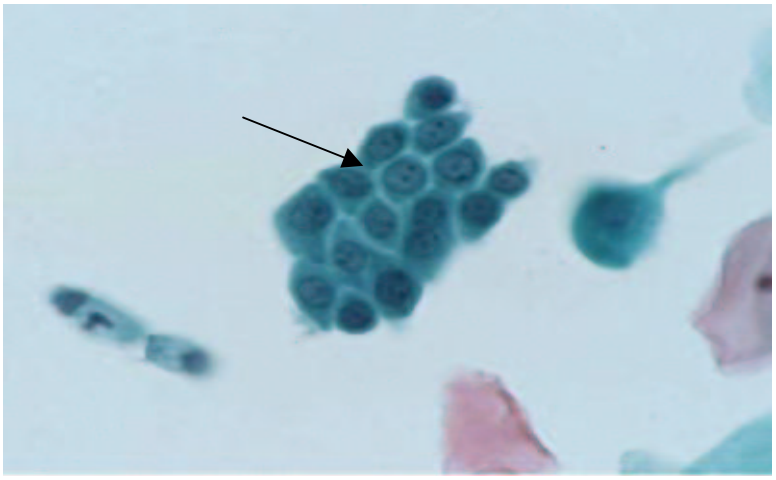


Figure 16: Satisfactory smear

(Endocervical cells)

(Taken from the Bethesda system for reporting cervical cytology)

"Unsatisfactory" smears that are processed and evaluated require considerable time and effort (Fig.17). Although such specimens cannot exclude an epithelial lesion, information such as the presence of organisms, or endometrial cells in women 40 years of age and older, etc., may help direct further patient management (Solomon and Nayar, 2003).

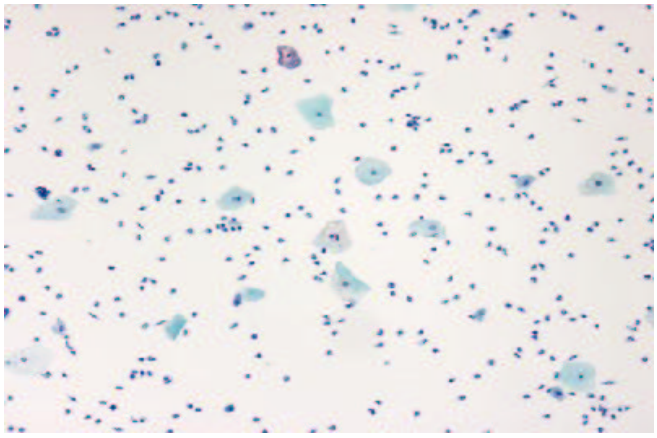


Figure 17: Unsatisfactory smear

(Note - too few squamous cells).

(Taken from the Bethesda system for reporting cervical cytology)

Negative for intraepithelial lesion or malignancy:

When there is no cellular evidence of neoplasia, but various fungi or viruses can be present (Figs. 18, 19, 20 and 21).

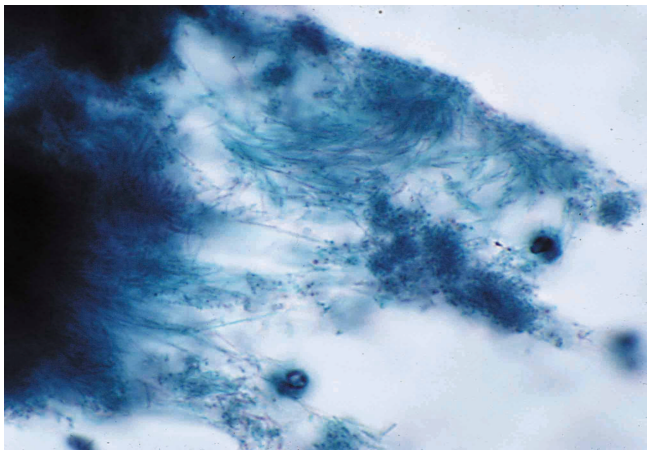


Figure 18: Actinomyces

(Note - filaments which can be found with women using an Intra-uterine contraceptive device)

(Taken from the Bethesda system for reporting cervical cytology)

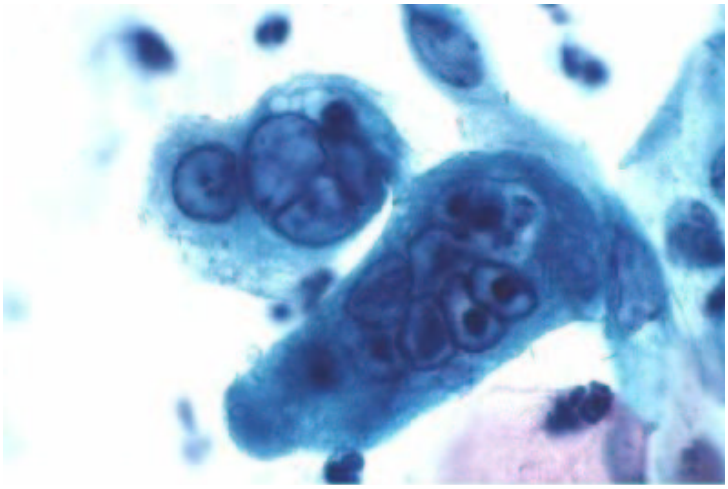


Figure 19: Herpes viral infection

(Note - intra-nuclear inclusions)

(Taken from the Bethesda system for reporting cervical cytology)

The virus that caused genital herpes is sexually transmitted. Although the virus cannot be seen using the light microscope the cytopathic effects (Fig. 19) it causes can be seen. The virus is surrounded by an envelope and contains DNA; it is 120nm in diameter and replicates in the nucleus of squamous cells. The effects that the virus causes are cell fusion, intranuclear inclusions, nuclear moulding and a glassy appearance to the nuclei.

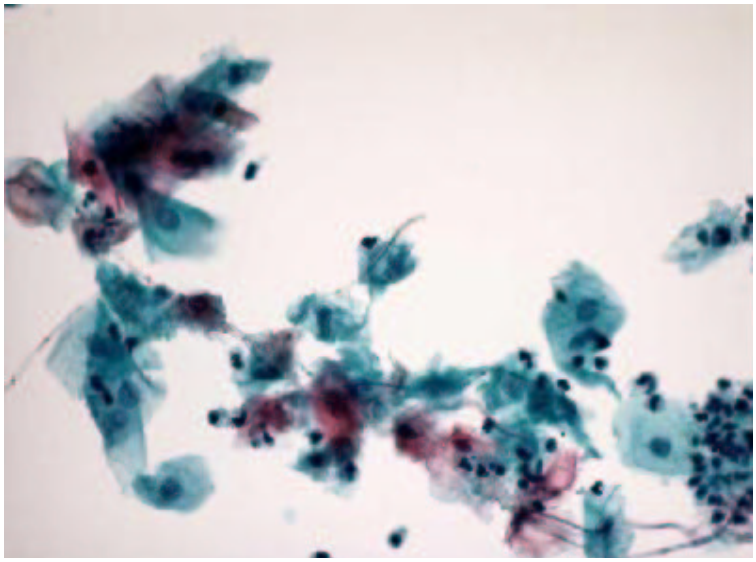


Figure 20: *Candida* species (pink filaments a fungal infection)

(Note squamous cells are normal and some reactive)

(Taken from the Bethesda system for reporting cervical cytology)

Candida albicans, is sometimes known as monilia, it is the cause of thrush. In a cervical smear monilia is seen as budding branching hyphae (Fig. 20) that are often intertwined with epithelial cells. *Candida albicans* prefers an acid pH and is often associated with lactobacilli, which produce lactic acid from the glycogen present in intermediate squamous cells. An inflammatory process is often seen.

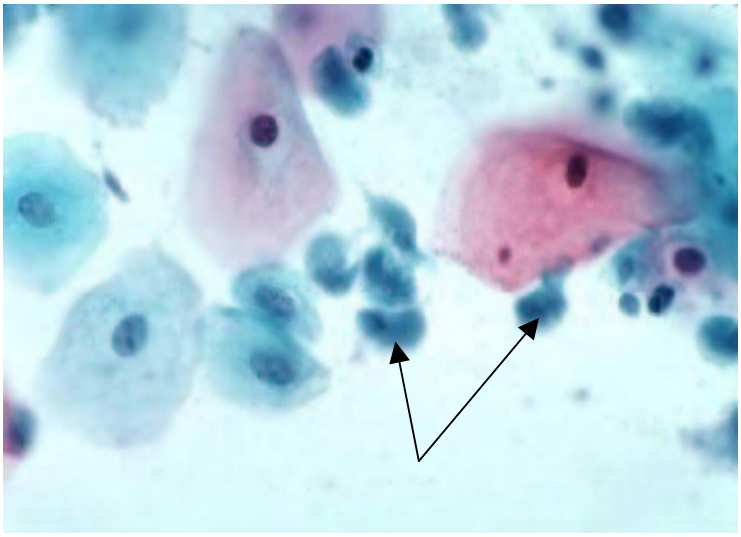


Figure 21: Trihomonas vaginalis

(Note – pear shaped organisms with eccentrically located nucleus and eosinophilic cytoplasmic granules. Squamous cells are normal and some reactive)

(Taken from the Bethesda system for reporting cervical cytology)

This is protozoan that causes a sexually transmitted infection. In its live state Trichomonas have two flagella, but these are destroyed by fixation. The organism stains a slate blue colour, (Fig. 21), sometimes showing red cytoplasmic granules, and contains an ellipsoid nucleus. It is usually present in large numbers and clusters around the edge of superficial squamous cells. The cells show an inflammatory response to the infection with maturation of the squamous cells. Enlarged and irregular nuclei with perinuclear halos may be seen.

Abnormal smears:

ASC-US: Criteria – The nuclei are approximately two and a half to three times the area of the nucleus of a normal intermediate squamous cell (Fig.22). There is minimal nuclear hyperchromasia and irregularity in the chromatin distribution or nuclear shape (Solomon and Nayar, 2003).

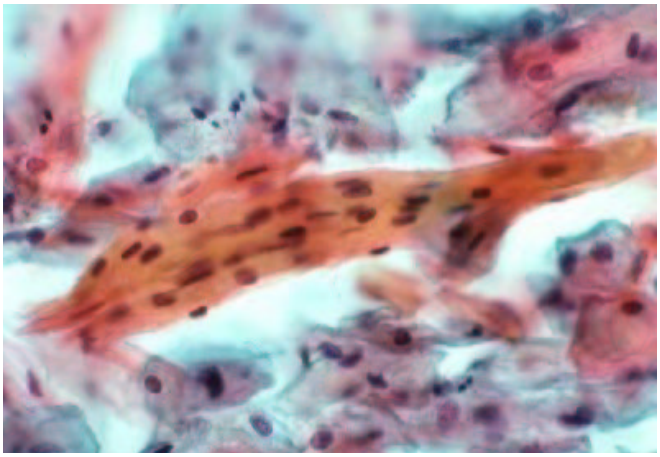


Figure 22: ASC-US

(Note - enlarged nuclei and orange cytoplasm)

(Taken from the Bethesda system for reporting cervical cytology)

ASC-H: Criteria – Cells usually occur singly or in small groups. The cells are the size of metaplastic cells (Fig. 23) with nuclei that are about one and a half to two and a half times larger than normal. The n/c ratio may approximate that of HSIL.

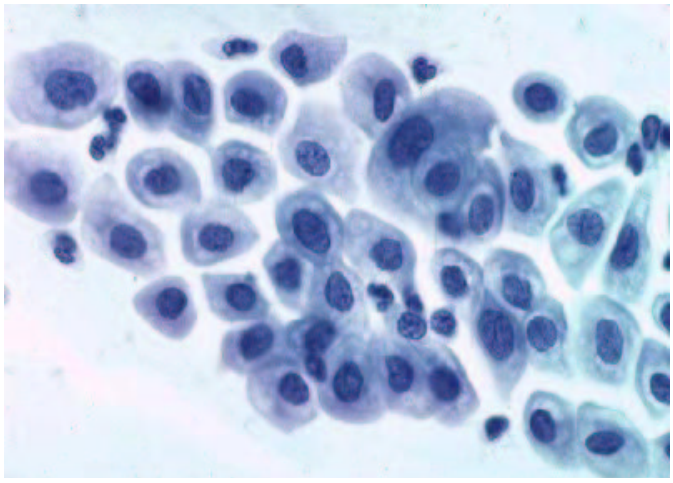


Figure 23: An example of ASC-H

(Note - small cells, the size of metaplastic cells and high n/c ratio)

(Taken from the Bethesda system for reporting cervical cytology)

LSIL: Criteria – The cells occur singly or in sheets. Cytologic changes are usually confined to cells with mature or superficial-type cytoplasm. Variable degrees of nuclear hyperchromasia are accompanied by variations in nuclear size, number, and shape. Binucleation and multinucleation are common. The cells have distinct cellular borders. Perinuclear cavitation ("koilocytosis"), consisting of a sharply delineated clear perinuclear zone (Fig. 24) and a peripheral rim of densely stained cytoplasm, is a characteristic feature but not required for the interpretation of LSIL (Solomon and Nayar, 2003).

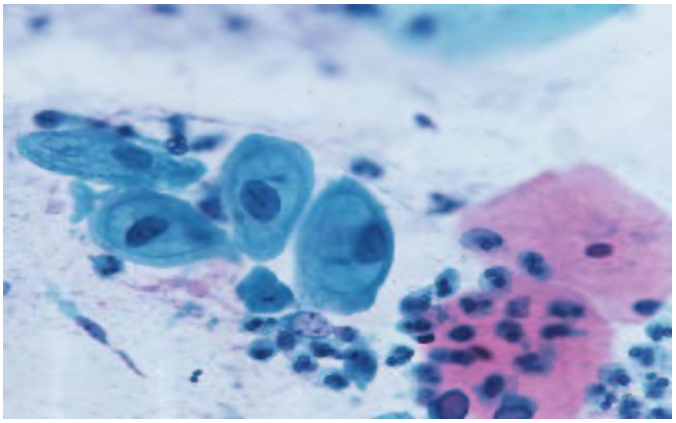


Figure 24: An example of LSIL (Note- the koilocytes)

(Taken from the Bethesda system for reporting cervical cytology)

Squamous Carcinoma: Criteria – The nuclei demonstrate markedly irregular distribution of coarsely clumped chromatin (Fig. 25). A tumor diathesis background consisting of necrotic debris and old blood is often present. There are different variants of squamous carcinoma.

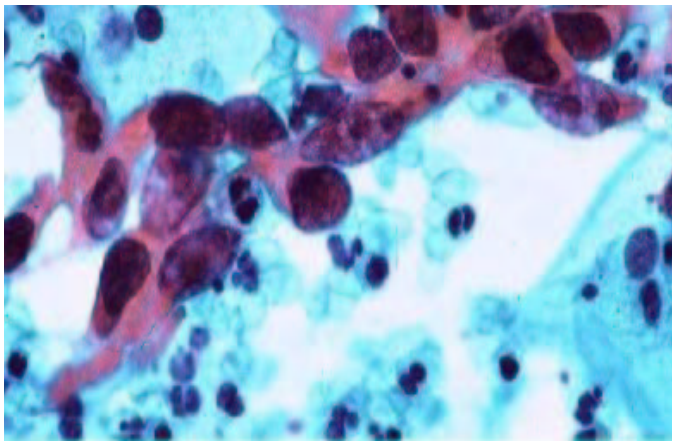


Figure 25: An example of Squamous carcinoma

(Note - the pleomorphic nuclei and prominent nucleoli)

(Taken from the Bethesda system for reporting cervical cytology)

Adenocarcinoma: Criteria – Adenocarcinoma is carcinoma of glandular epithelium, e.g., endometrium and endocervical. Cells occur typically in small, tight clusters. Variation in nuclear size and loss of polarity are evident (Fig. 26). The nuclei display moderate hyperchromasia, irregular chromatin distribution, and parachromatin clearing, particularly in high-grade tumors (Solomon and Nayar, 2003).

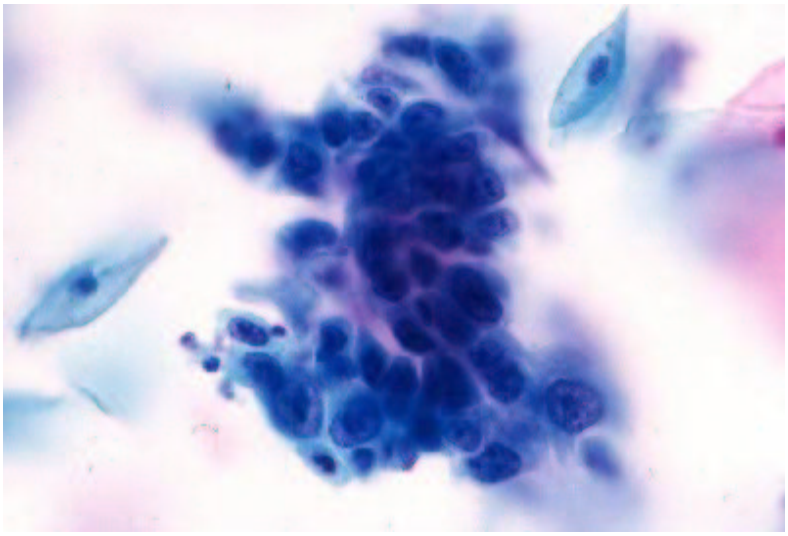


Figure 26: An example of Adenocarcinoma of the endocervix

(Note - the hyperchromasia and the nucleoli)

(Taken from the Bethesda system for reporting cervical cytology)

2.4.10.4 Consensus Guidelines for management:

Consensus Guidelines exist which was developed by ASCCP during a consensus meeting shortly after the Bethesda meeting.

Table 3: Definitions of terms in Consensus Guidelines (Taken from Apgar, *et al.*, 2003).

Definitions of Terms Utilized in the Consensus Guidelines

Colposcopy is the examination of the cervix, vagina, and, in some instances the vulva, with the colposcope after the application of a 3-5% acetic acid solution coupled with obtaining colposcopically-directed biopsies of all lesions suspected of representing neoplasia.
Endocervical sampling includes obtaining a specimen for either histological evaluation using an endocervical curette or a cytobrush or for cytological evaluation using a cytobrush.
Endocervical assessment is the process of evaluating the endocervical canal for the presence of neoplasia using either a colposcope or endocervical sampling.
Diagnostic excisional procedure is the process of obtaining a specimen from the transformation zone and endocervical canal for histological evaluation and includes laser conization, cold-knife conization, loop electrosurgical excision (i.e., LEEP), and loop electrosurgical conization.
Satisfactory colposcopy indicates that the entire squamocolumnar junction and the margin of any visible lesion can be visualized with the colposcope.
Endometrial sampling includes obtaining a specimen for histological evaluation using an endometrial biopsy or a "dilatation and curettage" or hysteroscopy.

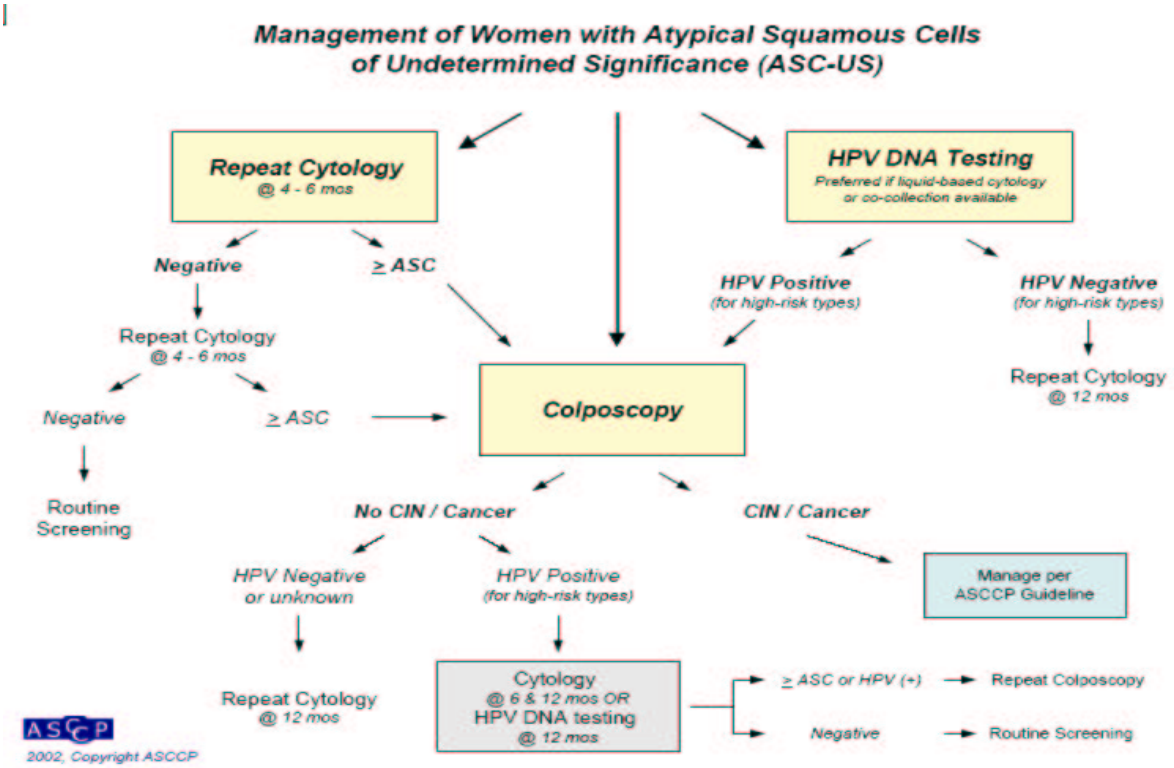


Diagram 2: Management of women with ASC-US (Taken from Apgar, *et al.*, 2003)

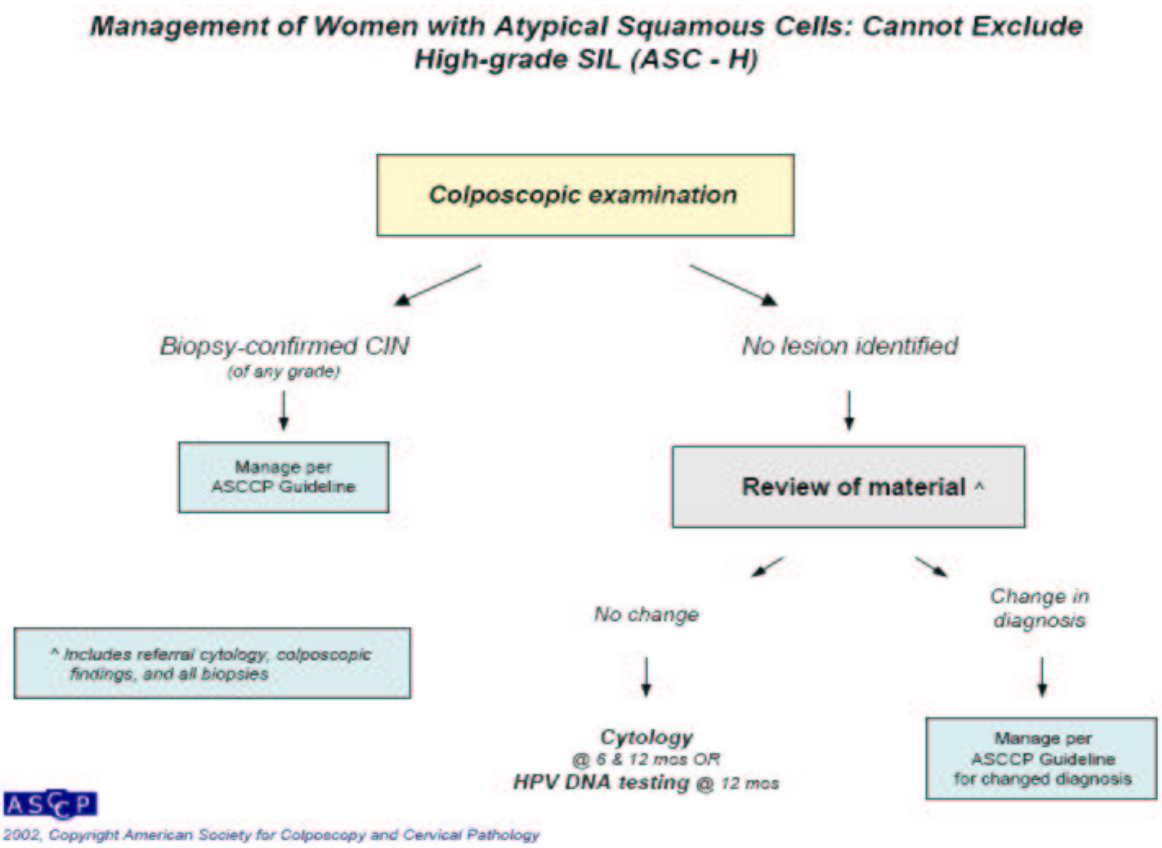


Diagram 3: Management of women with ASC-H (Taken from Apgar, *et al.*, 2003).

Management:

The new consensus management guidelines tailored to the 2001 Bethesda System classification recommend different follow-up for women with ASC-US as opposed to those with ASC-H.

Oncogenic (high-risk) HPV DNA testing is the preferred management for ASC-US when it can be performed concurrently with cytology; repeat cytological testing and immediate colposcopy also represent acceptable management.

In contrast, the recommended management of ASC-H following colposcopy that does not result in a histological diagnosis of CIN 2 or a more severe lesion should be individualized based on review of all pathologic and clinical findings.

Careful review is required before treating a woman with ASC-H who does not have histological diagnosed CIN 2 or worse (Solomon and Nayar, 2003).

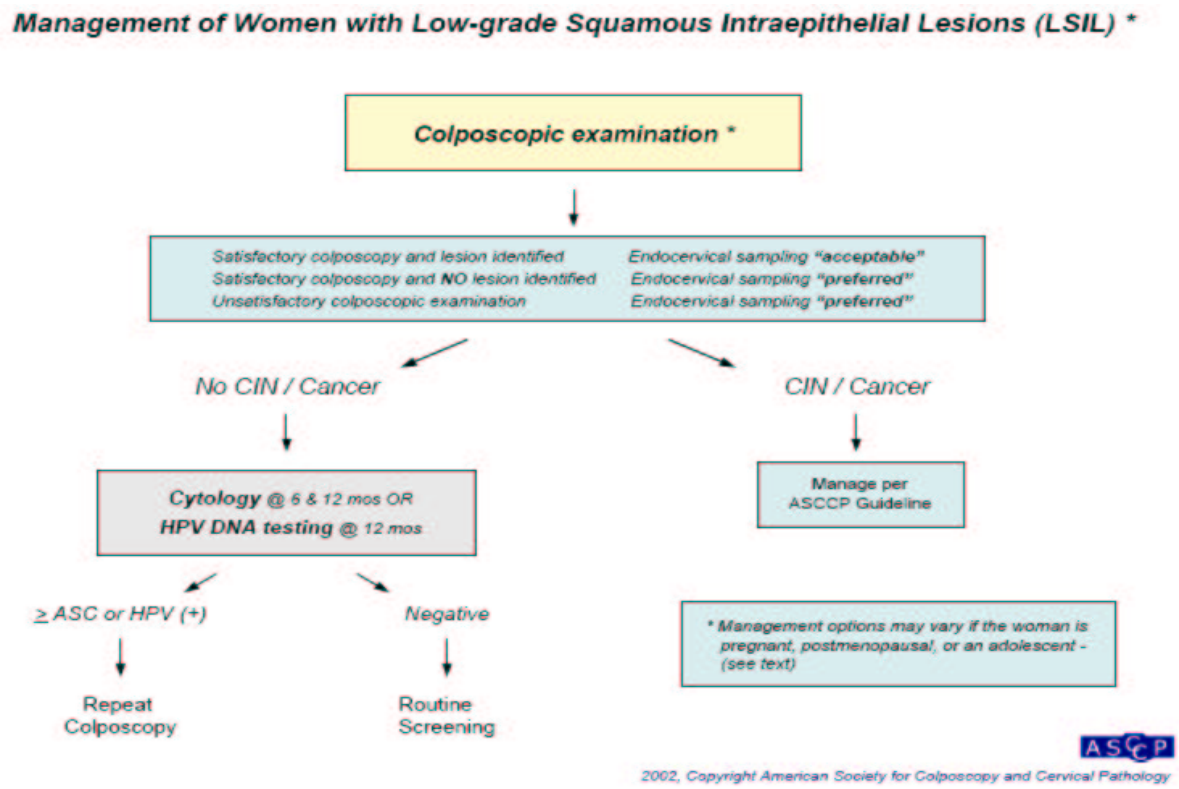


Diagram 4: Management of women with LSIL (Taken from Apgar, *et al.*, 2003).

Management of LSIL:

In data from the ASC-US/LSIL Triage study, high-risk HPV types were detected in 85% of LSIL cases, with the conclusion being that HPV testing is not a useful triage strategy. The ASCCP consensus guidelines recommend colposcopy for the initial management of LSIL.

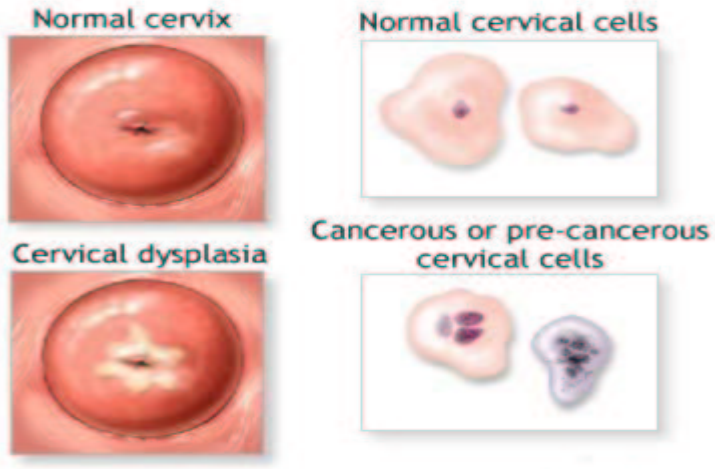


Figure 27: Colposcopic view of cervical dysplasia (Taken from http://www.nlm.nih.gov/medline)

HPV testing may have a potential role in the detection of persistent high-risk-type viral infection in patients with LSIL, especially because evidence has accumulated that persistent HPV infection is the major risk factor for "progression" (Solomon and Nayar, 2003).

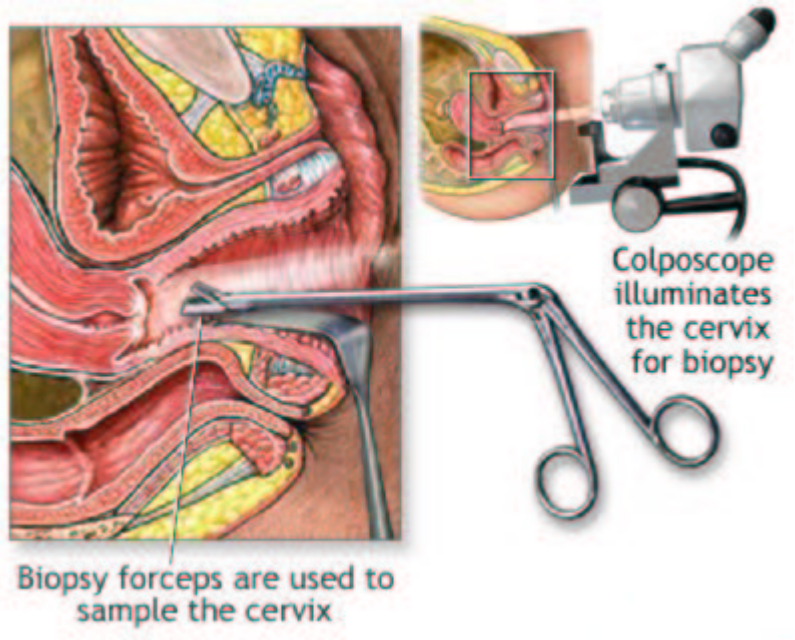
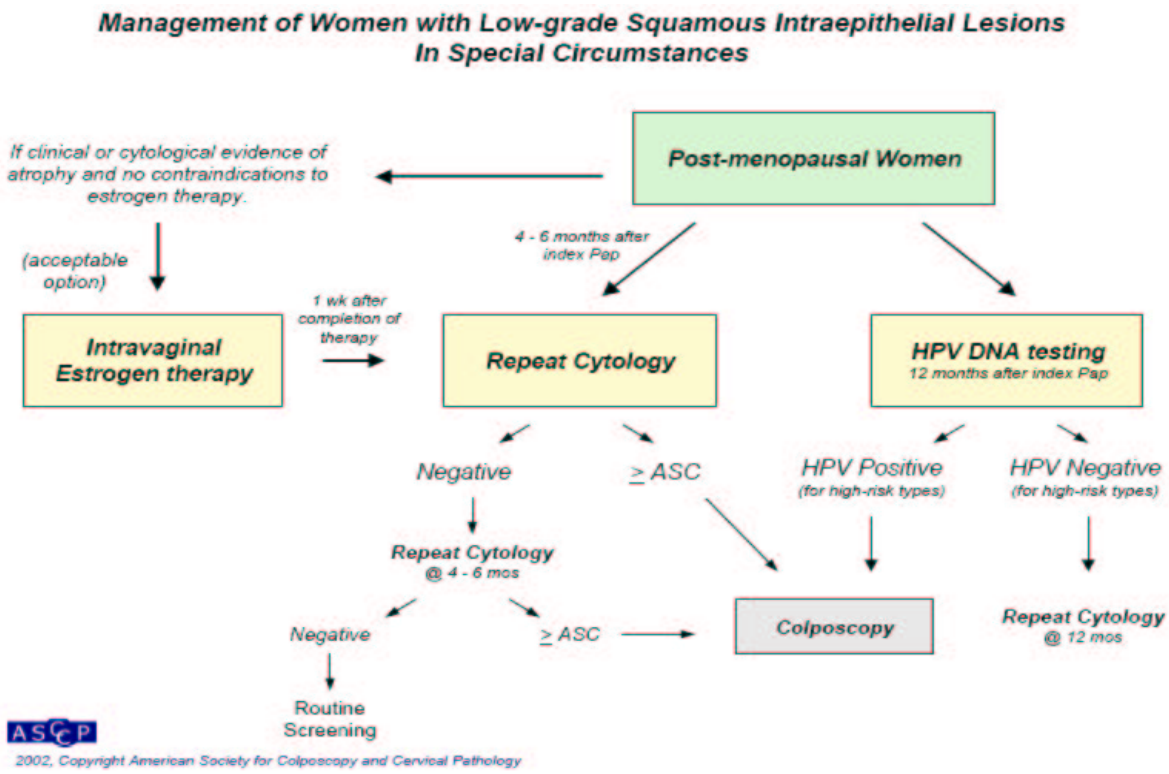


Figure 28: Sampling of cervix (Taken from http://www.nlm.nih.gov/medline)



Diag. 5: Management of women with LSIL in special circumstance (Taken from Apgar, *et al.*, 2003).

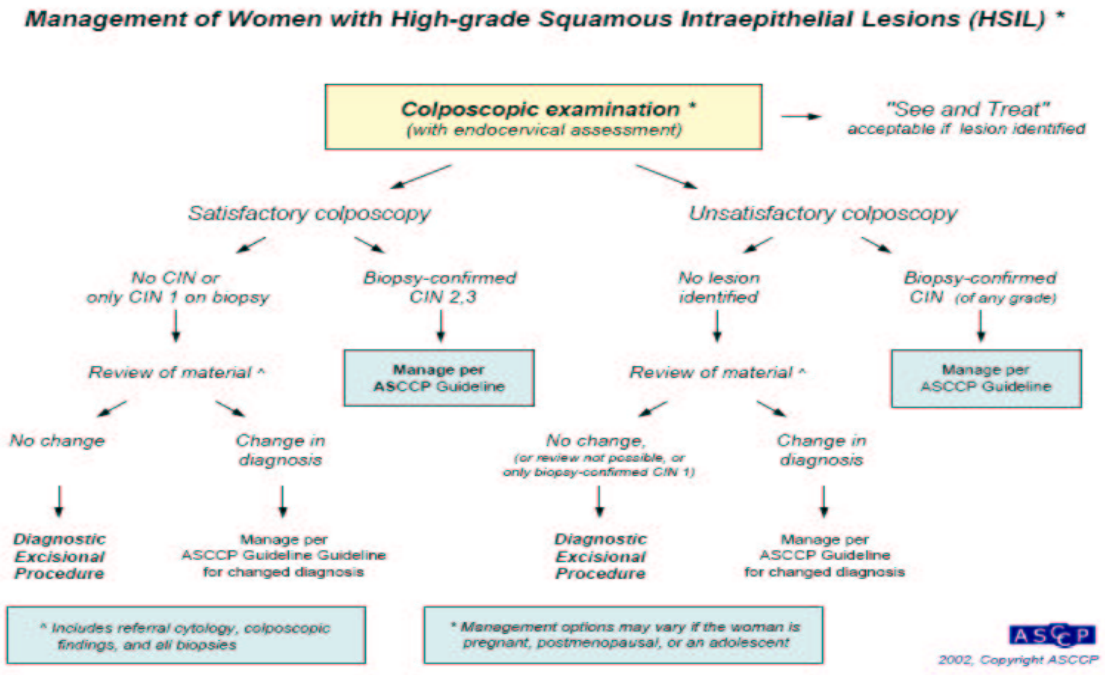


Diagram 6: Management of women with HSIL (Taken from Apgar, *et al.*, 2003).

Management of women with HSIL:

Most women with a cytological result of HSIL will have biopsy-confirmed CIN 2 or CIN 3 identified at the time of colposcopy (fig. 29).

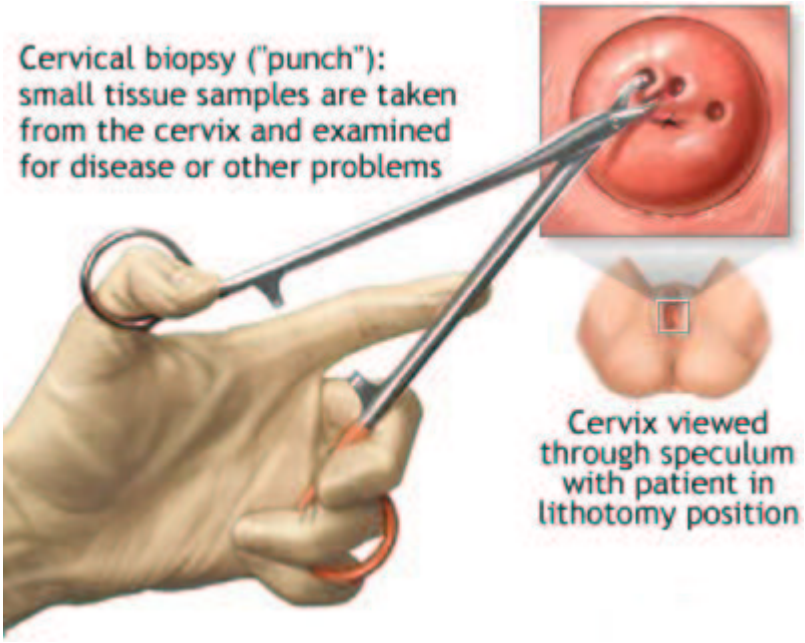


Figure 29: Cervical biopsy (Taken from http://www.nlm.nih.gov/medline)

Therefore, the 2001 ASCCP consensus guidelines recommend that if a biopsy-confirmed CIN is not identified at colposcopy in a woman with a cytological interpretation of HSIL, all cytological and histological material should be reviewed.

If the cytological interpretation of HSIL is upheld on review, a diagnostic excisional procedure should be performed (Solomon and Nayar, 2003)

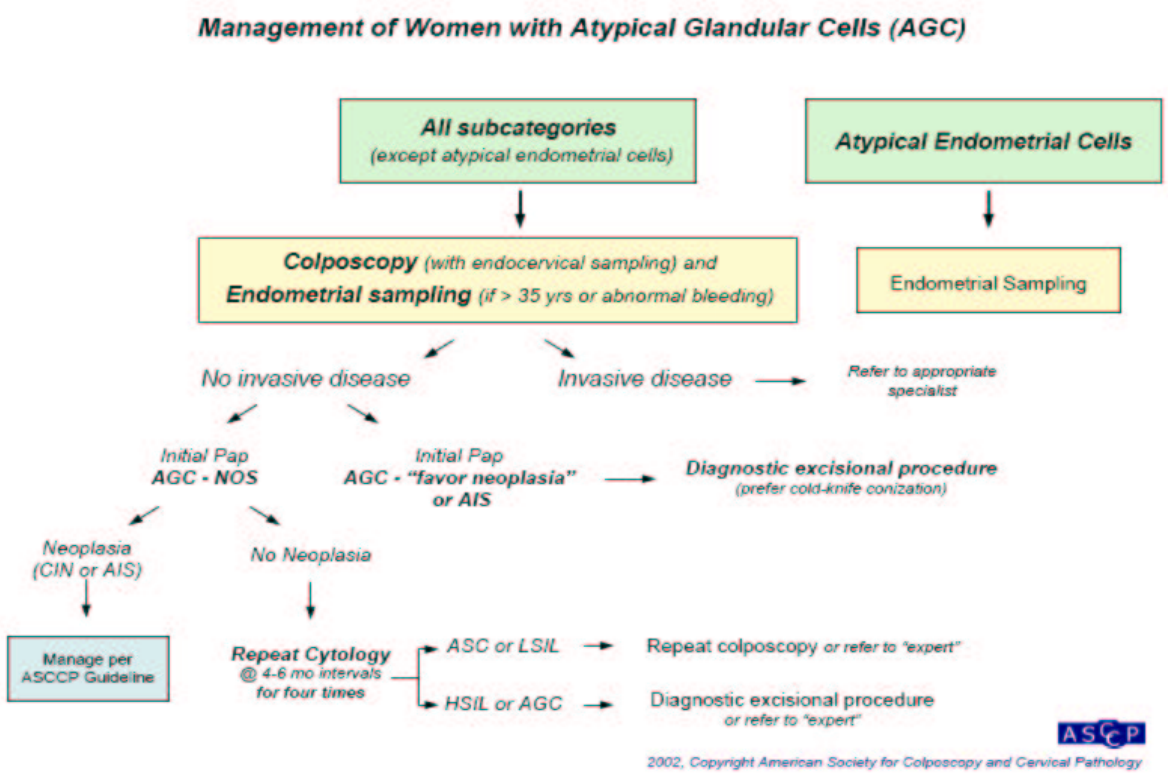


Diagram 7: Management of women with AGC (Taken from Apgar, *et al.*, 2003).

Management of women with AGC:

Colposcopy and endocervical and/or endometrial sampling are recommended. If the histological result does not compare with the cytological findings, these slides should be reviewed and discussed with the clinician. No abnormal cells on cytological findings can be ignored.

All cervical smears are screened and the above nomenclature and recommendations followed exactly (Apgar, *et al.*, 2003).

2.4.11 Typing:

The report is typed and handed to the pathologist.

Since 2001 we have implemented the Bethesda system in our laboratory and after many typing errors decided to design a worksheet with canned text in order to limit the typing errors. Free text can be used as and when necessary.

The implementation of this worksheet (Table 4) minimized typing errors as the screener marks the bold printed canned text, e.g. "neg", the typists type "neg" and it expands to “Negative for malignant cells.” In that way the whole report with the appropriate recommendations is marked or highlighted by the screener and the typists type it accordingly.

All diagnoses with their appropriate recommendations as per Bethesda system is incorporated into this canned text system. All screeners and pathologists therefore apply the Bethesda nomenclature and recommendations as per preset canned text. The screener writes the report number and the patient’s name at the bottom of the sheet and signs her name to identify the screener of the slide.

Staining, mounting and labeling is quality checked and marked as good or poor on the worksheet (Table 4). The typist who typed the report stamps her name and the pathologist concerned signs if need be. All relevant data is rechecked before the result is signed off and ready to be printed. The result is now ready to be posted or e-mailed (SOP-WI-PHIST-500).

This worksheet with the original request form is filed numerically.

All abnormal slides and request forms are kept indefinitely and all normal slides with their request forms for a minimum of 10 years.

Table 4: Example of worksheet (Taken from SOP-WI-PCYTO-008)

NATURE OF SPECIMEN

ROUTINE	PAP	CX SMEAR	VAULT	VAGINAL	ENDOPAP

PER A / B	PEP A / B	PEC A / B	PEV A / B	PEVAG A / B	PEND A / B

ADEQUACY

SATISFACTORY **SAT**	ENDOCX **ECC**	ENDOCX ABSENT **ECCA**	PART OBSC **PARTI** **PARTB**	POOR PRESERV **CELL**	UNSAT REJECTED **UNREJ**	UNSAT BECAUSE **UNSAT** ……

BACKGROUND

RBCELLS **R1 / R2 / R3** + / ++ / +++	NEUTS **N1 / N2 / N3** + / ++ / +++	NO INFLAM **PE43**

FLORA

TRICH **TR**	CANDIDA **C**	BACTERIAL SHIFT **BS**	BACT VAGINOSIS **BV**	ACTINOMYCES **A**	HERPES **H**	DODER **PEQD03**	CYTOLYSIS PEQD04

MORPHOLOGY

ASCUS	*ASCH*	*LSIL*	*HSIL*	*MICRO*	*SQ*

SQ NORMAL **N**	MIXED CELL PATTERN **MIXED**	SUPERF+ INTERM **SI**	INTERMED CELLS **I**	PARABAS INTERM+ **IP**	PARAB AS **PAR**

SQ REACT CHANGES **REACT**	METAPL **MMETA** **IMETA**	PARAKERAT SLIGHT/EXT **PEQE44.1 / PEQE44.2**	HYPERKERAT SLIGHT/EXT **PEQWC73.1 / PEQWC73.2**

GLANDULAR ABSENT **ABS**	GLANDULAR NORMAL **N**	GLANDULAR REACT **REACT**	ENDOMETS PRESENT **ENDOM**	AGC NOS **AGC**	AGC ENDOCX NOS **AGCX**
AGC ENDOM NOS **AGCE**	AGC FAV NEOPL **NAGC**	AGC ENDOCX FAV NEOPL **NAGCX**	ENDOCX IN SITU **AIS**	ADENOCA **AD**	ATYP EPITH CELLS **ATYP**

DIAGNOSIS

NEG	Negative	**C**	Candida
RASCUS	ASC-US first	**RBV**	Bacterial vaginosis
RASCP	ASC-US persistent	**RA**	Actinomyces
RASCH	ASC-H	**RKER**	Para/hyperkeratosis
RLSIL	LSIL	**RREP**	Repeat 4-6 wks
RHSIL	HSIL	**RPE13.2**	Endometrial cells + 40years
RAGC	AGC NOS	**REA**	Endocervical cells absent
RAGCX	AGC endocervical NOS	RCLIN	Follow-up as clinically indicated
RAGCE	AGC endometrial NOS	**STAINING**	**G P** **TYPED BY**

RNAGC	AGC favour NEOPLASIA	**MOUNTING**	**G P**	
RNAGCX	AGC endocervic favour NEOPLASIA	**LABELLING**	**G P**	
		NAME		
ATRO	Atrophic smear			
TR	Trichomonas vaginalis		**04**:	
H	Herpes			

2.4.12 Re-check:

To reduce the possibility of incorrect assessment of smears by the laboratory, we introduced in 2000 a quality control procedure for all negative and inadequate smears to be rescreened preferably prior to sending out such reports. These smears are currently subjected to shortened rescreening by a different member of staff, a method found to detect abnormalities missed on primary screening. The process, referred to as "rapid review", has proven to be helpful in identifying smears wrongly assessed initially as either adequate or inadequate, as well as for finding abnormalities (Cross, 1997). (Some laboratories also use this rapid review screening method to quickly assess a smear for on – site diagnoses. The slide is then later screened properly in the laboratory (Ditchmen, *et al.*, 1999).

Therefore, although the result is now ready and available to the doctor, (in our laboratory) EVERY smear is rescreened by the checker to ensure a 100% correct result. Once again the patient's history and clinical data is checked and any previous reports retrieved and checked according to the current slide and follow-up recommended.

When a recheck shows a difference in opinion that slide and form is submitted to the pathologist in charge, discussed with the screener, and monitored as such on the error list. Amendments and recommendations may be authorized by the pathologist in charge (SOP-WI-PCYTO-035).

2.4.13 Statistics:

The statistics of every screener as well as the average error rate of the laboratory is monitored constantly and discussed with the pathologist in charge.

Error rate is explained by the following flow diagram:

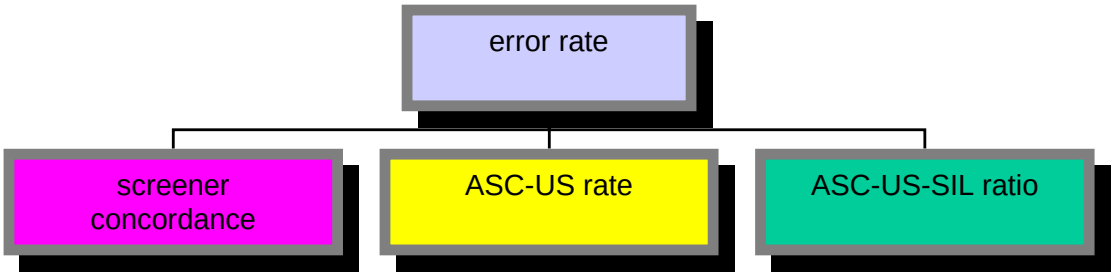


Diagram 8: Flow chart of error rate

2.4.13.1 Concordance rates:

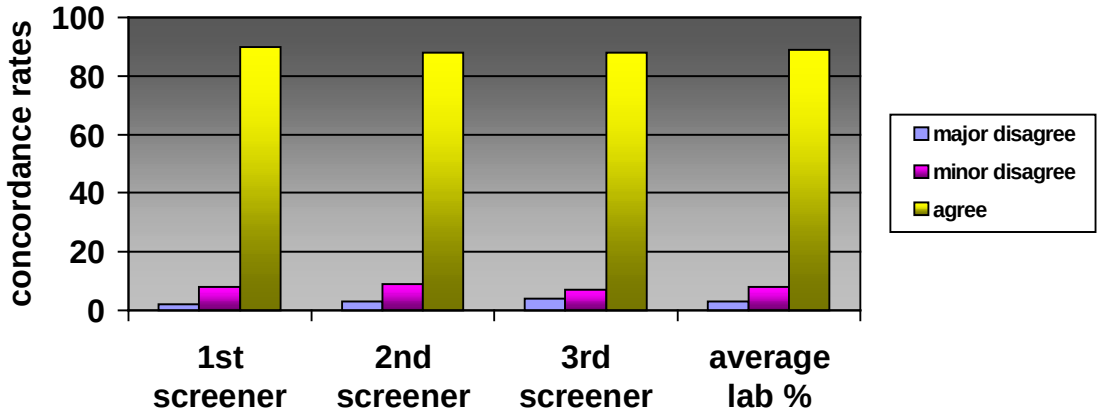


Figure 30: Screener concordance rates for the year 2003

(Data taken from Ampath laboratory Port Elizabeth)

ASC-US ratings and Error ratings are compiled and completed on a monthly basis and then discussed with the pathologists as well as the screeners.

Table 5: ERROR RATE 2002 (Data taken from Ampath laboratory Port Elizabeth)

MONTH	1ST SCREENER %	2ND SCREENER %	3RD SCREENER %	LAB AVERAGE	NO.OF ABNORMAL SMEARS RECHECKED
JANUARY	4,8	1,76	1,69	2,75	266
FEBRUARY	2,5	1,25	0	1,85	101
MARCH	3,8	3,4	1,11	2,7	315
APRIL	4,4	2,6	2,5	3,25	312
MAY	3,0	3,75	4,1	3,18	264
JUNE	3,6	3,3	5,6	4,1	241
JULY	3,8	3,4	2,24	4,2	335
AUGUST	5,17	4,16	2,5	3,85	177
SEPTEMBER	0	1,75	3,85	1,41	183

OCTOBER	4,65	3,03	3,84	3,84	167
NOVEMBER	2,86	4,47	3,2	3,72	160
DECEMBER	0	3,3	3,5	2,27	63
	3,22	3,01	2,84	3,09	2584

Table 6: ERROR RATE 2003 (Data taken from Ampath laboratory Port Elizabeth)

MONTH	**1ST SCREENER %**	**2ND SCREENER %**	**3RD SCREENER %**	**LAB AVERAGE**	**NO.OF ABNORMAL SMEARS RECHECKED**
JANUARY	3,1	1,59	1,64	2,11	159
FEBRUARY	0	1,4	1,56	0,99	169
MARCH	2,44	1,64	0	1,36	163
APRIL	2,63	1,85	2,7	2,39	168
MAY	2,32	1,54	1,72	1,86	169
JUNE	5,4	3,1	2,7	3,73	180
JULY	4,5	5,7	1,1	3,8	211
AUGUST	2,4	2,4	1,1	1,96	218
SEPTEMBER	1,72	0	1,37	1,03	182
OCTOBER	1,69	2,2	0	1,3	182
NOVEMBER	2	1,59	2,5	2,03	197
DECEMBER	0	10	12	7,3	136
	2,35	2,75	2,37	2,49	2134

The error rates per month as per Tables 5 and 6 are calculated per screener and as per average for the laboratory. This serves as a quality assurance measure and for the pathologist to observe when and where additional training is required (George, *et al*., 2004).

2.4.13.2 ASC-US-Rate and ASC-US-SIL Rate:

ASC-US Rate: The percentage of ASC-US results should not exceed 5% of the total of smears screened, when a high-risk population is screened.

If the rating in the laboratory exceeds this ratio, this should be discussed immediately as it may be an indication of overcalling pathology (false positives and low specificity.) ASC-US **should not be used as a collective basket for lack of making a better diagnosis.**

Table 7: **ASC-US rate (**Data taken from Ampath laboratory Port Elizabeth)

MONTH	2001	2002	2003	2004
JANUARY		2,75%	2,11%	2,63%
FEBRUARY		1,85%	0,99%	3,5%
MARCH		2,7%	1,36%	1,7%
APRIL		3,25%	7,18%	2,3%
MAY		3,18%	1,86%	2,82%
JUNE		4,2%	3,73%	3,35%
JULY		3,85%	3,8%	4,1%
AUGUST	5,2%	1,41%	1,96%	4,14%
SEPTEMBER	3,9%	3,84%	1,03%	2,23%
OCTOBER	5,16%	3,72%	1,3%	1,45%
NOVEMBER	1,0%	2,27%	2,03%	1,89%
DECEMBER	0,79%	3,12%	7,3%	2,42%
Annual average	**3,21%**	**3,0%**	**2,89%**	**2,71%**

The percentages in Table 7 show that the ASC-US rate in the laboratory is within the set limits.

ASC-US – SIL rate: **The ratio of ASC-US to LSIL + HSIL should not be more than**

2-3:1

Table 8: **ASC-US - SIL RATE 2002 RATIO SHOULD NOT BE MORE THAN 2-3:1**

(Data taken from Ampath laboratory Port Elizabeth)

MONTH	ASC-US	L-SIL	HSIL	RATIO	NO. OF SLIDES
JANUARY	**29**	**38**	**21**	**0,50:1**	**1594**
FEBRUARY	**47**	**36**	**18**	**0,87:1**	**1737**

MARCH	**112**	**27**	**11**	**2,90:1**	**1569**
APRIL	**114**	**55**	**7**	**1,80:1**	**1514**
MAY	**60**	**36**	**11**	**1,22:1**	**1527**
JUNE	**102**	**30**	**7**	**2,75:1**	**1363**
JULY	**73**	**29**	**8**	**2,02:1**	**1366**
AUGUST	**51**	**28**	**11**	**1,30:1**	**1486**
SEPTEMBER	**31**	**22**	**6**	**1,10:1**	**1290**
OCTOBER	**34**	**29**	**5**	**1,00:1**	**1603**
NOVEMBER	**21**	**27**	**8**	**0,60:1**	**1419**
DECEMBER	**22**	**11**	**3**	**1,57:1**	**983**

Table 9: ASC-US - SIL RATE 2003 RATIO SHOULD NOT BE MORE THAN 2-3:1

(Data taken from Ampath laboratory Port Elizabeth)

MONTH	ASC-US	L-SIL	HSIL	RATIO	NO. OF SLIDES
JANUARY	**33**	**28**	**10**	**0,87:1**	**1545**
FEBRUARY	**36**	**38**	**12**	**0,72:1**	**1695**
MARCH	**39**	**42**	**13**	**0,71:1**	**1531**
APRIL	**31**	**28**	**12**	**0,78:1**	**1344**
MAY	**34**	**45**	**13**	**0,59:1**	**1732**
JUNE	**32**	**30**	**7**	**0,86:1**	**1303**
JULY	**39**	**47**	**5**	**0,75:1**	**1425**
AUGUST	**30**	**29**	**7**	**0,83:1**	**1561**
SEPTEMBER	**38**	**28**	**14**	**0,90:1**	**1350**
OCTOBER	**53**	**37**	**11**	**1,10:1**	**1520**
NOVEMBER	**27**	**29**	**11**	**0,68:1**	**1393**
DECEMBER	**42**	**31**	**8**	**1,07:1**	**905**

The ratios in column 5 of both Tables 8 and 9 prove that the ASC-US-SIL rate is well within its normal limits at the Ampath laboratory Port Elizabeth.

2.4.13.3 Follow-up letters

Follow-up letters (Table 10) are also sent to submitting health professionals as a reminder to follow up patients with abnormal smears (Shuter, 2003).

Table 10: Example of follow-up letter (Data taken from Ampath laboratory Port Elizabeth)

Dr. XYZ					
October 2004					
Date	Number	File number	Name	Diagnosis	Recommendation
12/10/2004	CS1234	56	Mrs. ABC	LSIL	Colposcopy
November 2004					
Date	Number	File number	Name	Diagnosis	Recommendation
2/11/2004	CS5678	78	Mrs. DEF	ASC-US	Colposcopy / Repeat smear
December 2004					
Date	Number	File number	Name	Diagnosis	Recommendation
5/12/2004	CS9876	89	Mrs. GHI	LSIL	Colposcopy
14/12/2004	CS1357	12	Mrs. JKL	ASC-US	Colposcopy / Repeat smear

2.4.13.2 Turn around time

The time from when a specimen is received in the laboratory until the result is sent out is logged. Turn around time is checked twice a month. The turn around time for the laboratory concerned is 6 hours. According to SANAS regulations, all reports should be on the doctor's desk within 5 days.

2.4.14 Comparison with histology specimens:

All cervical biopsies are checked for previous cervical smear results, and *visa versa* (Karateka, *et al*., 2004). When the results differ, the pathologist requests the rescreening of the appropriate cytology slides and if he agrees with the result, he may ask for deeper

sections of the biopsy or sometimes even requests for the biopsy to be rotated and be sectioned from the other side (Anderson, *et al*., 2004). The pathologist will then mention in his report what the outcome of the comparison of cytology and histology is and make recommendations accordingly (SOP-WI-PHIST-096).

Cyto-histological correlation may be performed prospectively at the time of histological review. The review may also be performed retrospectively. All negative cervical cytology smears of the specific patient must be reviewed when a new HSIL or carcinoma is detected on smears by cytology or histology (SOP-WI-PHIST-096).

2.5 CONCLUSION:

Having read multiple articles, journals and books regarding the 14 points above, the author came to the conclusion that it will not be very cost effective to endeavour to screen a higher population of South African women, since there are large rural areas which do not have easy access to primary health care. To get these patients to come to the urban areas for colposcopies, biopsies or any other recommended follow-up, seems barely possible and the average woman will definitely be lost for follow-up (own observation).

Although screening of every female patient at least once in a lifetime is preferable in an ideal world, this is not practical due to financial and other constraints. Therefore energy should be directed to at least ensuring optimal evaluation and management of the 41% of patients who do present for cervical screening (own observation).

CHAPTER 3

RESEARCH DESIGN AND METHODOLOGY

3.1 Study design

All cervical smears received since the year 2000 to 2004 (n = +/- 61 000) will be retrieved from the archives of the Ampath Cytology laboratory Port Elizabeth.

With the following three objectives in mind, all these smears will be evaluated and re-evaluated accordingly:

- To determine whether the presence or absence of endocervical component has an effect on the adequacy of cervical smears
- To determine the impact of smaller coverslips on quality assurance in the cytology laboratory
- Evaluate the effect that re-screening of smears has on quality assurance in the cytology laboratory.

3.2 Data collection

In this section a brief discussion will be given with regard to the data collection process, including techniques and procedures used.

3.2.1 Transformation zone component:

All results on all cervical smears in our laboratory since the year 2000 (n = +/- 61 000) were retrieved from the archives of the Cytology Ampath laboratory in Port Elizabeth and the presence or absence of transformation zone component calculated.

3.2.2 Impact of size of coverslips on quality assurance:

All abnormal cervical smears since the year 2000 (n = 5 347) were retrieved and the smaller coverslip measured manually on the slide. The smaller coverslip was placed over the area of best concentration of cells.

Therefore the smaller coverslip were sometimes placed near the frosted end of the slide and other times in the middle of the slide or at the end of the slide – depending on the way the smear was spread on the slide.

3.2.3 Effect of 100% rescreening of slides on quality assurance:

There are various methods of rescreening slides for quality assurance. Literature shows that various methods have been tried and that each one has its own positive and negative points (Adab, *et al*., 2004). Most laboratories implement the 10% quality control, which means that the checker chooses any one of ten smears, and rescreen this slide as quality control. The author was concerned about the other 90% not being checked.

Other laboratories also implemented the scan method where the slide is scanned in a step-up and step-down manner or even scanned under a 4 X objective and only thicker areas on a 10 X objective or 40 X objective if need be (own observation).

The use of a rapid rescreening method as part of internal quality control was implemented in the year 2000. This entails the full rescreen of slide, but in a shorter time than normal. Literature differs, some requesting a one-minute rapid rescreen and others a 2-minute rapid rescreen per slide (Farrell, *et al.*, 1997).

The author takes every slide on its own merits. If the slide is very bloody or very cellular with many inflammatory cells, more time will be spent on this slide opposed to a slide with less cells and with a clear background. When the patient has had a previous abnormal diagnosis or abnormal clinical findings, this slide will also be rescreened more carefully (Cross, 1997).

A total of 55 583 cervical smears of 48 785 patients with a result of Negative for malignancy were rescreened.

3.3 Data analysis

Statistical analysis of the data was undertaken. Some results are given in numbers and others as a percentage using the Microsoft Excel software package.

CHAPTER 4

RESULTS

4.1 Presence or absence of transformation zone component:

The following studies were done in our own laboratory from the year 2000 to 2004.

Table 11: Smears counted with present or absent transformation zone

(Data taken from Ampath cytology laboratory Port Elizabeth)

	No of smears Counted	Neg. smears Transformation zone absent	Neg. smears Transformation Zone present	Abn. smears Transformation zone absent	Abn. smears Transformation Zone present
2000	3 468	1204	2141	28	95
2001	12 657	4129	7600	204	724
2002	15 669	4870	9412	336	1051
2003	15 751	4847	9369	371	1164
2004	13 387	4193	7818	296	1080
Total	60 932	19243	36340	1235	4114

2000:

As per Table 11 – of the 3 468 smears counted**,** 1 204 (34, 72%) had negative results with absent transformation zone, and 2 141 (61, 74%) had negative results with present transformation zone.

Twenty-eight (0, 8%) smears had abnormal results with absent transformation zone, and 95 (2, 74%) smears had abnormal results with present transformation zone.

2001:

As per Table 11 – of the 12 657 smears counted, 4 129 (32, 62%) had negative results with absent transformation zone, and 7 600 (60, 05%) had negative results with present transformation zone.

Two hundred and four (1, 61%) smears had abnormal results with absent transformation zone, and 724 (5, 72%) smears had abnormal results with present transformation zone.

2002:

As per Table 11 – of the 15 669 smears counted, 4 870 (31, 08%) had negative results with absent transformation zone, and 9 412 (60, 07%) had negative results with present transformation zone.

Three hundred and thirty-six (2, 14%) smears had abnormal results with absent transformation zone, and 1 051 (6, 71%) smears had abnormal results with present transformation zone.

2003:

As per Table 11 – of the 15 751 smears counted, 4 847 (30, 77%) had negative results with absent transformation zone, and 9 369 (59, 48%) had negative results with present transformation zone.

Three hundred and seventy-one (2, 36%) smears had abnormal results with absent transformation zone, and 1 164 (7, 39%) smears had abnormal results with present transformation zone.

2004:

As per Table 11 – of the 13 387 smears counted, 4 193 (31, 32%) had negative results with absent transformation zone, and 7 818 (58, 40%) had negative results with present transformation zone.

Two hundred and ninety-six (2, 21%) smears had abnormal results with absent transformation zone, and 1 080 (8, 07%) smears had abnormal results with present transformation zone.

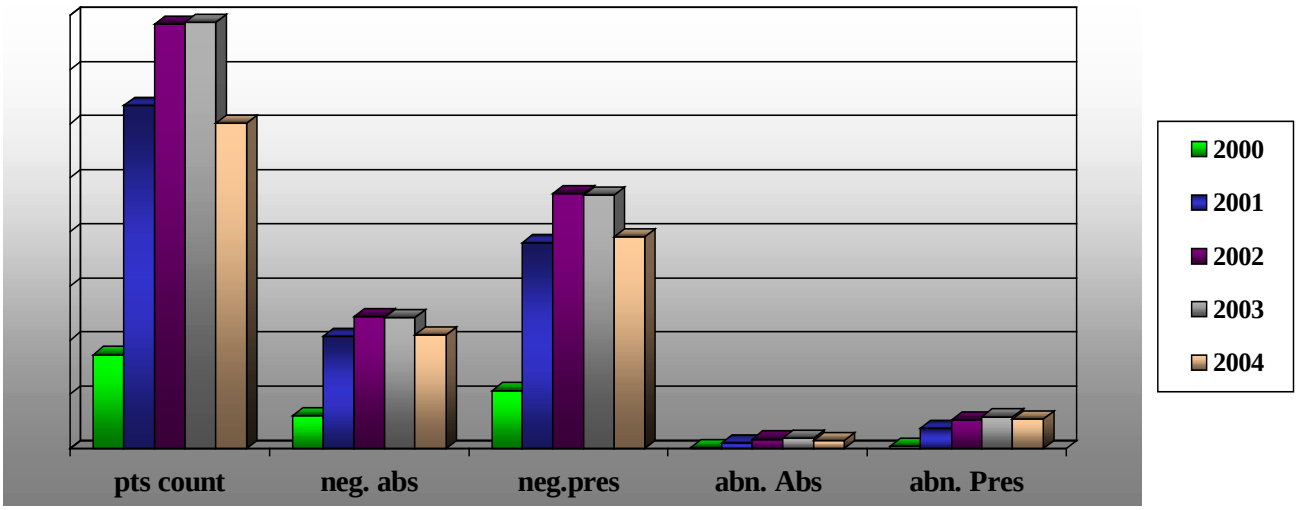


Figure 31: Patients counted with present or absent transformation zone
(Data taken from cytology laboratory Ampath Port Elizabeth)

As per Table 11 and Figure 31 a total of 60 932 smears were scanned.

Of these smears, 36 340 (59, 64%) were negative for malignant cells with present transformation component and 4 114 (6, 75%) smears had abnormal results with present transformation component.

Of the smears counted, 19 243 (31, 58%) smears had negative results with absent transformation component and 1 235 (2, 03%) smears had abnormal results with absent transformation component.

The total percentage of smears with absent component = 33, 61% (Table. 11).

4.2 Impact of size of coverslips on quality assurance:

All abnormal cervical smears were retrieved and the smaller coverslip measured manually on the slide. The smaller coverslip was placed over the area of best concentration of cells.

Therefore the smaller coverslip were sometimes placed near the frosted end of the slide and other times in the middle of the slide or at the end of the slide – depending on the way the smear was spread on the slide.

Table 12: Abnormal smears assessed with smaller coverslips

Abnormal Smears counted		No of slides with abnormal cells outside small coverslip border	Ratio of slides with cells outside border: total slides	Percentage of abn. cells outside small coverslip border
2000 (1 semester)	123	9	9: 123	7,32%
2001	928	63	63: 928	6,79%
2002	1387	52	52: 1387	3,75%
2003	1533	56	56: 1533	3,65%
2004	1376	43	43: 1376	3,13%
Totals	5347	223	223:5347	4,17%

2000:

As per Table 12 - of the total number of 123 patients with abnormal results counted in the second semester of the year 2000**,** 9 smears showed abnormal cells outside the edge of

the smaller coverslip. This is 7, 32% of the abnormal smears screened in the second semester of the year 2000 that would not have been diagnosed as abnormal.

2001:

Of the total number 928 patients with abnormal results counted in the year 2001, 63 smears showed abnormal cells outside the edge of the smaller coverslip. This is 6, 79% of the abnormal smears screened in the year 2001 that would not have been diagnosed as abnormal.

2002:

Of the total number 1 387 patients with abnormal results counted in the year 2002, 52 smears showed abnormal cells outside the edge of the smaller coverslip. This is 3, 75% of the abnormal smears screened in the year 2002 that would not have been diagnosed as abnormal.

2003:

Of the total number 1 533 patients with abnormal results counted in the year 2003, 56 smears showed abnormal cells outside the edge of the smaller coverslip. This is 3, 65 % of the abnormal smears screened in the year 2003 that would not have been diagnosed as abnormal.

2004:

Of the total number 1 376 patients with abnormal results counted in the year 2004, 43 smears showed abnormal cells outside the edge of the smaller coverslip. This is 3, 13% of the abnormal smears screened in the year 2004 that would not have been diagnosed as abnormal.

From the year 2000 – 2004, 4, 17% of all abnormal smears had abnormal cells only outside the edge of the smaller coverslip and would therefore have had false-negative results.

This is a total of 223 smears that would have been diagnosed as false negative.

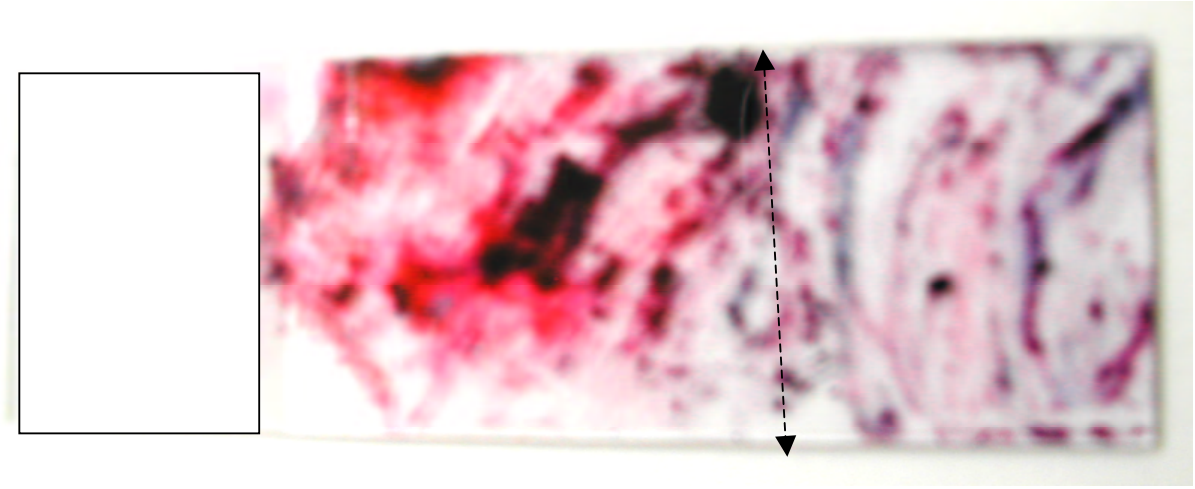


Figure 32: Ecto- and endocervical smear on one slide
(Data taken from cytology laboratory Ampath Port Elizabeth)

Taking into consideration the full size of the slide (76mm x 26mm), the frosted end measures 20mm x 26mm. The rest of the slide measures 56mm x 26mm. A large coverslip of 50mm x 24mm covers thus 82 % of the slide and the smaller coverslip measuring 40mm x 24mm covers thus 66 % of the slide. An adequate smear consists of an average of at least 8000 to 12000 squamous cells (normally up to 500 000 cells).

Due to cost saving, (Fig. 32), more and more clinicians actually place the endocervical smear on one half of the slide and the ectocervical smear on the other half. In this way the whole slide is covered and sometimes some clinicians even put their smears over the frosted end of the slides.

The golden rule with mounting always applies – the coverslip must be placed over the area with the most concentration of cells.

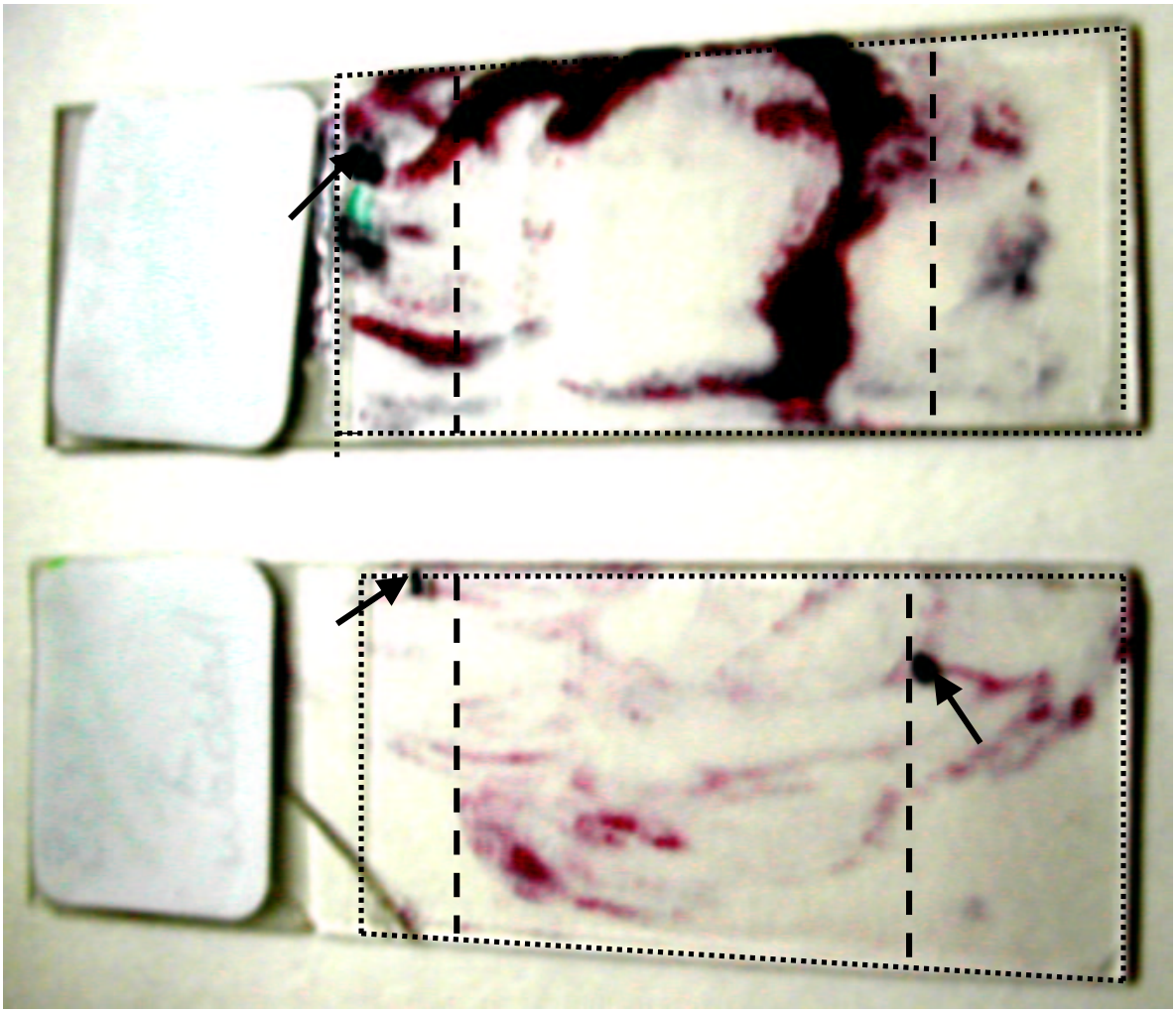


Figure 33: The effect of smaller coverslips

(Data taken from cytology laboratory Ampath Port Elizabeth)

50 x 24 mm coverslip

40 x 22 mm coverslip

The black markings (high-lighted with arrows) on both slides in Figure 33, shows that should a smaller coverslip have been used; the abnormal cells would have been missed, resulting in a false negative result.

In our laboratory, 223 smears would definitely been diagnosed as false negative, if the smaller coverslip was in use.

4.3 Effect of 100% rescreening of slides on quality assurance:

The use of a rapid rescreening method (rescreening the entire smear for at least 1 – 2 minutes) as part of internal quality control was implemented in the year 2000 (SOP-WI-PCYTO- 035).

A total of 48785 patients (Table 13) with 55 583 smears with negative cervical smear results were rescreened (some patients have more than one smear.)

Table 13: Number of patients and percentages with agreements and disagreements

	No of patients	Minor disagreements		Major disagreements		Agreed upon	
2002 1 semester	18213	24	(0, 13%)	9	(0, 05%)	18180	(99, 82%)
2003	17304	27	(0, 16%)	11	(0, 06%)	17266	(99, 78%)
2004	13268	13	(0, 10%)	7	(0, 05%)	13248	(99, 85%)
Total	48785	64	(0, 14%)	27	(0, 05%)	48694	(99, 81%)

Rapid rescreening was established in the Ampath laboratory Port Elizabeth in 2000 following the publication of several studies indicating improved sensitivity for the detection of abnormalities in cervical smears. During the study period, 2002 – 2004, as per Table 13, 48 785 patients with negative results were rapidly rescreened. A total of 91 patients (0, 19%) were identified with incorrect results and selected for full rescreening.

Of these, 91 cases were considered inaccurate following pathologist review and resulted in a corrected report or an amended report.

Of these, 29 cases were confirmed as ASC-US (Fig. 34).

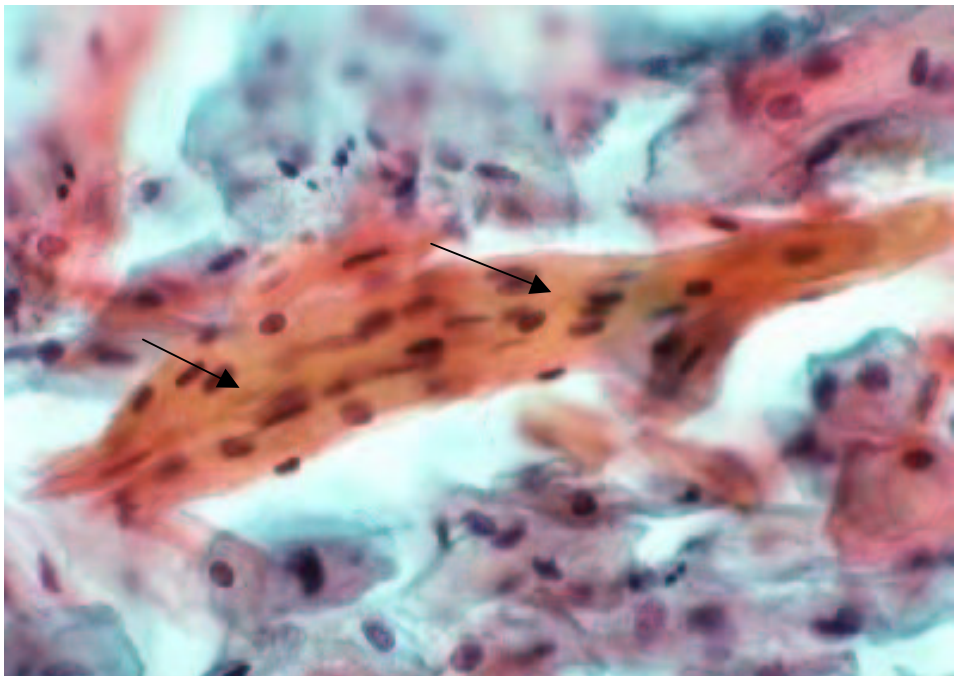


Figure 34: An example of ASC-US

(Taken from the Bethesda system for reporting cervical cytology)

The nuclei of the orange streak of cells are approximately two and a half to three times the area of the nucleus of a normal intermediate squamous cell (Fig.34). There is minimal nuclear hyperchromasia and irregularity in the chromatin distribution or the nuclear shape (Solomon and Nayar, 2003). These cells can easily be misinterpreted if not evaluated very carefully.

Seven cases were confirmed as ASC-H (Fig. 35).

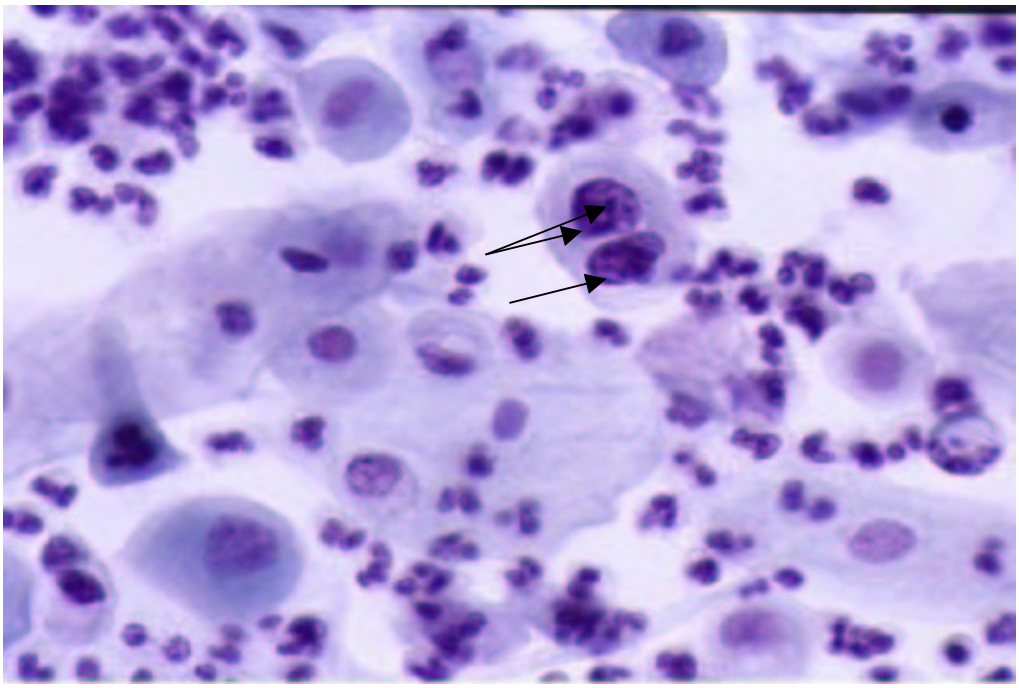


Figure 35: An example of ASC-H – note two small atypical cells

(Taken from the Bethesda system for reporting cervical cytology)

The ASC-H cells are the two small cells just off centre towards two o'clock.

ASC-H cells usually occur singly or in small groups. The cells are the size of metaplastic cells (Fig. 35) with nuclei that are about one and a half to two and a half times larger than normal. The n/c ratio may approximate that of HSIL. The nuclear membranes are irregular and sometimes indented.

These cells are small and are sometimes mistaken for degenerate squamous metaplastic cells or atrophic parabasal-like cells.

Nine cases were confirmed as LSIL (Fig. 36).

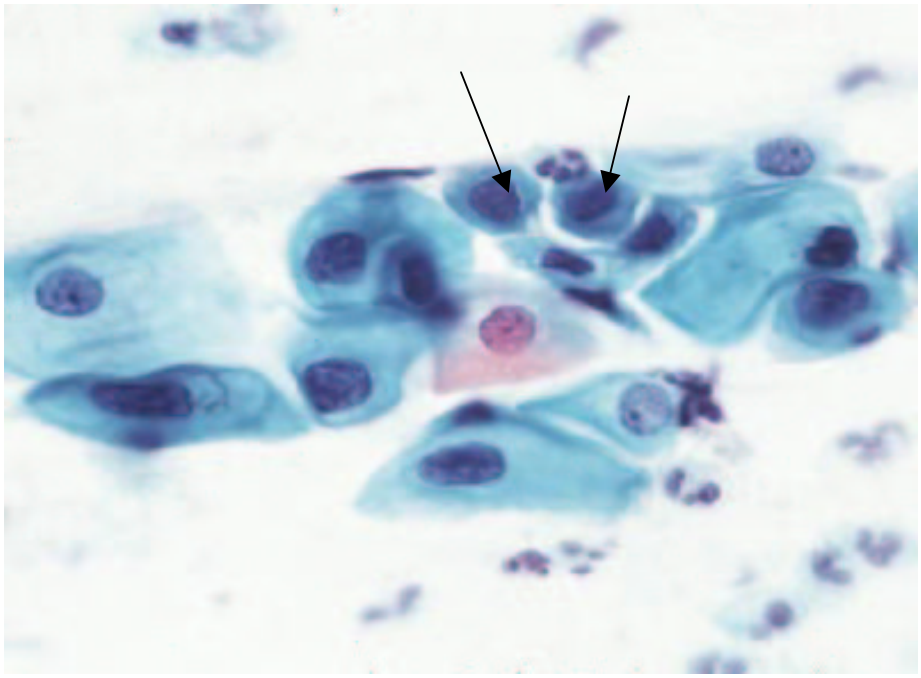


Figure 36: An example of a smear with LSIL

(Note – some smaller cells suggestive of HSIL)

(Taken from the Bethesda system for reporting cervical cytology)

The cells occur singly or in sheets. Cytological changes are usually confined to cells with mature or superficial-type cytoplasm. Variable degrees of nuclear hyperchromasia are accompanied by variations in nuclear size, number, and shape. The nuclear membrane is irregular and sometimes demonstrates prominent indentations or grooves. Binucleation and multinucleation are common. The cells have distinct cellular borders. Perinuclear cavitation ("koilocytosis"), consisting of a sharply delineated clear perinuclear zone (Fig. 36) and a peripheral rim of densely stained cytoplasm, is a characteristic feature but not required for the interpretation of LSIL (Solomon and Nayar, 2003).

Eleven cases were confirmed as HSIL (Fig. 37).

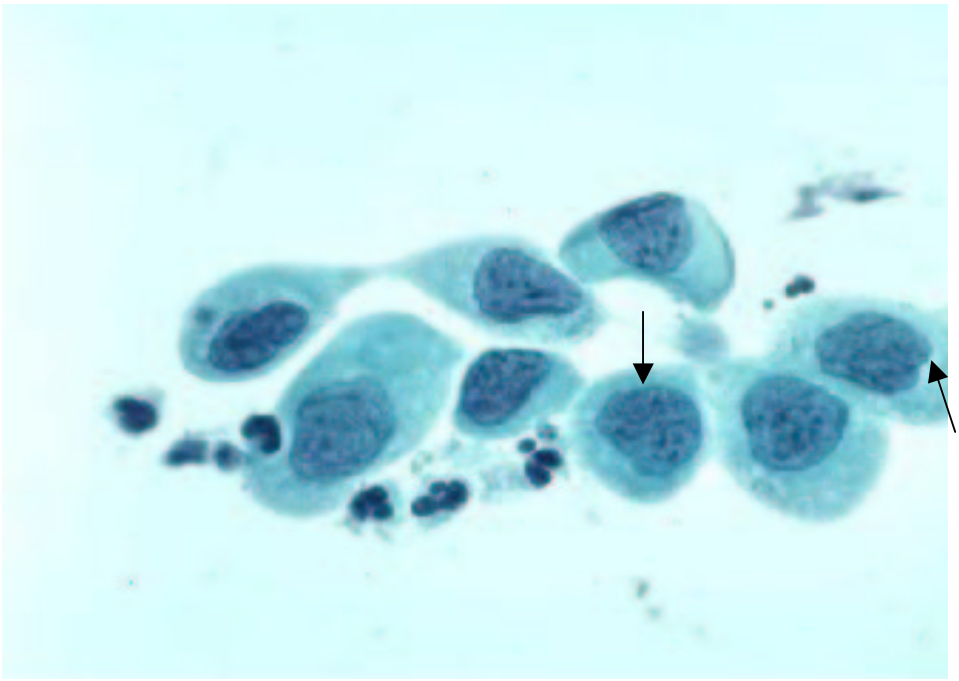


Figure 37: An example of a cervical smear with HSIL

(Taken from the Bethesda system for reporting cervical cytology)

Cytological changes affect the cells that are smaller and less "mature" than the cells in LSIL. The cells occur singly, in sheets, or in syncytial-like aggregates. Nuclear hyperchromasia is accompanied by variations in nuclear size and shape. There is a marked increase in n/c ratio. The chromatin may be fine or coarsely granular and evenly distributed. The contour of the nuclear membrane is quite irregular and frequently demonstrates prominent indentations or grooves (Solomon and Nayar, 2003).

A type of HSIL, where the abnormal cells are rather scattered singly than in groups or syncitial arrangements, can evade rapid rescreening as well as routine screening (Fig. 37). This suggests that even when rapid rescreening is used as a quality assurance measure, the "zero-error standard" is unlikely to be attained.

Thirty-five results were changed in view of minor errors, i.e. wrong recommendations, organisms or viral infections present or lack of clinical data supplied (Fig. 38).

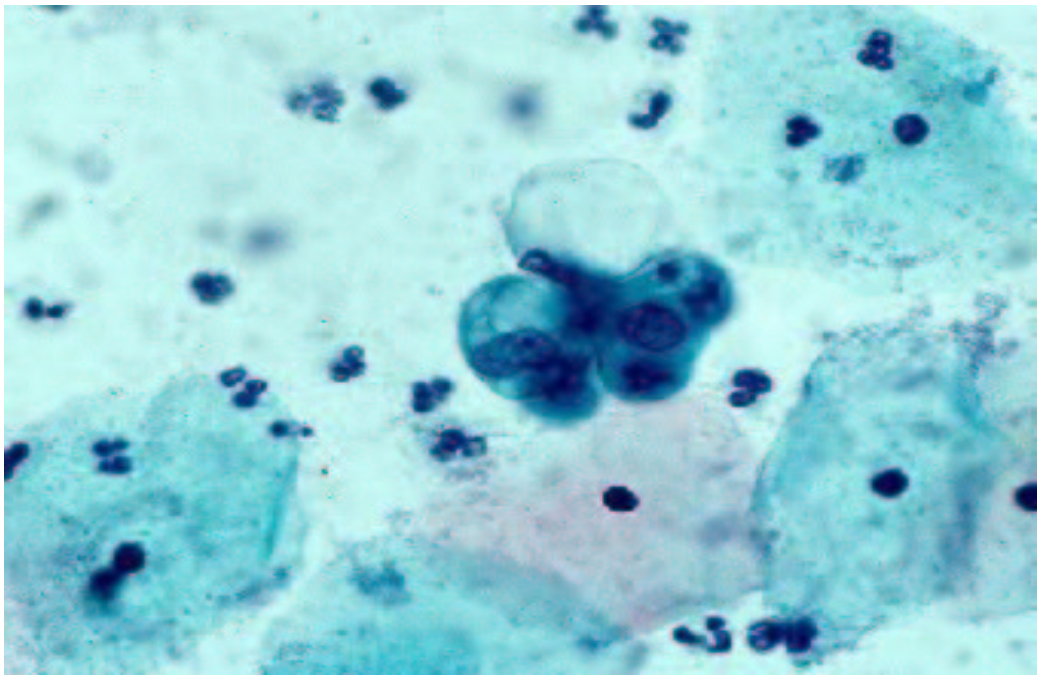


Figure 38: An example of very reactive endometrial cells due to an IUCD
(Taken from the Bethesda system for reporting cervical cytology)

Figure 38 demonstrates a group of vacuolated cells, some with macro-nucleoli. In the absence of clinical details, these cells can be diagnosed as atypical glandular cells. It was confirmed by the doctor that this patient has an IUCD, which explains these reactive and atypical looking endometrial cells.

Rapid screening of unscreened smears could also be used as means of selecting patients for prompt referral when a laboratory backlog exists. This happens in a very busy laboratory or where the laboratory is under-staffed.

The situation occurs when the screeners cannot keep up with the volume of work and a backlog of smears arises in the laboratory. The more urgent smears are then screened first and as the clinician phones for a result, a screener can quickly scan a smear and give the clinician a provisional diagnosis, then rescreen smear at own leisure. A printed or telephonic result may then be rendered.

Cytology laboratories that evaluate large numbers of slides per week may make use of automated screener devices as discussed in Chapter 3. These machines are used for primary screening or/and as secondary screening for quality control.

CHAPTER 5

DISCUSSION

5.1 Presence or absence of transformation zone component:

The question of adequacy of cervical smears is central to the success of cervical screening in the prevention of cervical cancer deaths. The problem of establishing exactly what constitutes an adequate sample has received increasing attention in recent years, culminating in various broad guidelines.

The Bethesda system of terminology advocates a mandatory statement on quality of smears; grouping smears as adequate and inadequate. While there is by no means unanimity over these criteria, it is important that cytologists have a degree of confidence about the threshold of acceptability of smear quality.

The feature of paramount importance in assessing smear quality is that there should be adequate numbers of epithelial cells on the slide, (8000 – 12 000 well visualized squamous cells and 10 squamous metaplastic cells or endocervical cells) with evidence that they are from the appropriate area of the cervix. In theory, the latter requirement can only be satisfied if squamous metaplastic cells, endocervical cells and mucus are present in sufficient proportions to establish that the entire circumference of the transformation zone has been scraped.

Clearly, however, metaplastic cells will not be firmly identifiable once the transformation zone is fully mature, and endocervical cells will not always be sampled, especially after the menopause.

Recognition of a representative sample therefore requires knowledge of the woman's age and menstrual status and of any hormonal treatment.

A smear with an abnormal result is always classified as "satisfactory" even if no transformation zone component is present.

As per Table 11 (Chapter 4) a total of 60 932 smears and 59 554 patients were scanned (some patients have more than one smear.) Of these smears, 36 340 (59, 64%) had negative for malignant cells with present transformation component results and 4 114 (6, 75%) smears had abnormal results with present transformation component.

Of the 60 932 smears counted, 19 243 (31, 58%) had negative results with absent transformation component results and 1 235 (2, 03%) smears had abnormal results with absent transformation component.

The total percentage of smears in the cytology laboratory Ampath Port Elizabeth with absent component results = 33, 61% (Table.11 – Chapter 4).

The author is concerned about the 19 243 (31, 58%) smears with negative results and absent transformation component. Our laboratory has a monthly follow-up reminder system in place. This letter is delivered to every clinician concerned. Unfortunately the clinicians alternate their work amongst the cytology laboratories in our area and therefore accurate follow-up records cannot always be maintained. As mentioned before, HSIL and Adenocarcinoma (and its precursors), occur higher up in the endocervical canal. The concern is thus the lesion-related errors arising from not taking smears with adequate endocervical or transformation zone sampling.

At the laboratory concerned, all negative for malignant cells smears with absent endocervical or transformation zone component, except atrophic smears, vault or vaginal smears, has recommendations for the management of these patients. The recommendation for absent component does not require a repeat smear immediately, unless the patient had

high-risk HPV results within 12 months, previous squamous abnormality without 3 subsequent negative cervical smears of which at least one contained endocervical component, a previous cervical smear with unexplained glandular abnormality, immunosuppression as well as insufficient previous screening. A repeat smear is

normally advised after an interval of 4 to 6 weeks to enable the epithelium some time to replenish. A postpartum smear is preferred, but not mandatory for pregnant patients.

A representative smear sample from the ecto- as well as the endocervix is thus required (own observation).

Cross sectional studies have repeatedly demonstrated that smears with endocervical cells have a significantly higher frequency and high grade of squamous epithelial abnormalities detected than do smears lacking such cells (Apgar, *et al.*, 2003).

In a study of 635 patients with previous abnormal cytology referred to colposcopy, Henry and Solomon (1995) examined the effect of the amount of endocervical component on the detection of squamous lesions. The presence of one to five endocervical cell clusters increased the number of HSIL cases detected from 6% to 10%. The presence of greater numbers of endocervical cells had no additional effect on the detection rate of HSIL. Interestingly, the presence of endocervical cells did not affect the detection rate of LSIL. In fact, large numbers of endocervical cells actually decreased the rate of detection of LSIL from 21% when one to five clusters were present, to 10% when greater than 25 cell clusters per slide were present. This may be a reflection of sampling too far up into the endocervix, thus missing lower grade lesions which are more commonly found on the ectocervix.

Obtaining cells from the transitional zone is important, because this is where most cervical dysplasia is seen**.** Several factors may affect the yield of endocervical cells: sampling technique; prior cervical procedures; surgical absence of the cervix; oral contraceptives; hormonal status of the patient (the transformational zone may be high within the endocervical canal) and pregnancy (Apgar, *et al.*, 2003).

Experts disagree on the management of the patient who lacks endocervical cells on cervical smear. It is recommended that routine follow-up at prescribed intervals is satisfactory without an endocervical component if: there is no suspicion of a

premalignant lesion; there is nothing of concern in the patients clinical history; there is no abnormality identified on physical examination; there is no history of a previous abnormal cervical smear and if there are no other clinical risk factors (DiSaia and Appleby, 2005).

Although compelling, these findings are tempered by observations indicating that specimens without an endocervical / transformation zone component nevertheless often contain diagnostic cells (Apgar, *et al.,* 2003). These cells are thought to be probably originating more from the ectocervix rather than the upper endocervical canal (own observation).

5.2 Impact of size of coverslips on quality assurance:

Conventional cervical smears are spread onto the slide by the smear taker, and keeping in mind the cost of cervical smear screening as well as the number of cells on the spatula, the smear taker has to transfer as many cells as possible onto the slide – as evenly as possible.

The smear taker can therefore use one half of the slide for an ectocervical smear and the other half of the same smear for an endocervical smear. This is where the problem of the coverslip sizes arises. As the author has proven in the findings, the larger coverslip only covers 82% of the slide, whereas the smaller coverslip used in this study only covers 66% of the slide.

This is a difference of 16% of the smear surface being covered and evaluated cytologically.

Taking into consideration that up to 300 000 cells may be on one smear, 16% of this smear can add up to 48 000 cells not being coverslipped and evaluated cytologically (own observation).

Figure 32 (Chapter 4) clearly shows that the two halves of the smear differ macroscopically. The doctor placed both the endocervical and ectocervical smear onto one slide to save costs for the patient. It is therefore quite obvious that a 50 x 24 mm coverslip could not cover the whole smear (own observation). The 40 x 24 mm smaller coverslip used in this study would therefore not have covered another 10mm of the smear, which could easily have contained the only abnormal cells on the smear.

Figure 33 (Chapter 4) illustrates the 2 black markings on the top slide and the 2 black markings on the bottom slide. The rule with using a smaller coverslip is to place this coverslip where the best concentration of cells is on the smear. It is therefore quite obvious that these abnormal cells would have been missed, had a smaller coverslip been used (own observation).

From the year 2000 – 2004, 4,17% of all abnormal smears at the Ampath laboratory in Port Elizabeth, had abnormal cells only outside the edge of the smaller coverslip and would therefore have had false-negative results.

This is a total of 223 smears that would have been diagnosed as false negative.

In 1998, the St Bartholomew's Hospital in London examined 923 smears covered by 40 x 22 mm size coverslips, inside and outside the coverslip area to determine whether this coverslip size could be responsible for missed abnormal cells in conventional cervical screening. There was no instance when abnormal cells seen outside the coverslip were not also present within the coverslipped area (Oommen, *et al.,* 1998). Unfortunately the authors did not mention the size of the area of the smears on the slides examined, as Figure 27 (Chapter 4) illustrates endo- and ectocervical smears on one smear covering the greater part of the slide.

When the Thin-prep or AutoCyte method of preparing a smear as a liquid-based specimen is used, the size of the coverslip will not matter, as the cells are all spun down onto the slide into a small circle in the middle of the slide. The smaller coverslip (some are round) will thus cover all the cells.

The National Association of Cytologists at the Hereford laboratory in England, agrees that the smaller coverslip (40mm x 24mm) definitely 'loses' 25% of the cells on the smear. David Procter (2000), points to their shortage of staff, the fact that all the cellular material is not transferred from the sampling device, as well as the screening errors due to the thousands of cells evaluated per screener per day. The author thinks that every medical practitioner has to take the responsibility of his/her part of the quality assurance trail. The screener can therefore not hide behind the fact that maybe some of the cells were not transferred to the slide, or maybe the lesion was not properly visualized therefore it does not matter to use smaller coverslips or make screening errors.

Cytology laboratories world-wide vary with the size of coverslips they use, whether manual mounting or an automated mounting machine is used. The author has proven the positive influence of using the larger coverslip for conventional smears, on proper quality assurance and correct diagnoses to which every patient is entitled.

Unfortunately, the method of smear taking or preparing is not always mentioned in the literature when the size of coverslips are discussed. It is therefore sometimes difficult to draw an accurate conclusion.

At the Ampath laboratory Port Elizabeth, most smears cover the entire non-frosted area of the slide and therefore require the use of the larger coverslip. The workload of the screener is increased by the use of the larger coverslip, but the quality assurance of each smear may not be compromised in any way (own observation).

5.3 Effect of 100% rescreening of slides on quality assurance:

Rescreening for quality control purposes should be conducted in a manner that recapitulates the laboratory's day-to-day operations as closely as possible. The slow, exaggerated rescreening that often takes place under testing or investigative circumstances might exaggerate the error rate, and will try to measure a type of performance other than that actually taking place.

This reaches its peak when slides are reviewed for medico-legal purposes and one is monetarily compensated for lavishing enormous amounts of time on a single cervical smear. One can look at the slide until cells that could never be detected by a technologist screening under normal laboratory circumstances are identified. Review of slides in this manner bears no relationship to actual practice. As a quality control methodology, it is misleading. As an expert witness's behaviour, it is probably unethical (Geisinger, K 2003).

This study shows that rapid rescreening definitely helps in lowering the false negative rate in the laboratory – with no additional effect on human resources. The technique also identifies variation in the performance of screening personnel as an additional benefit, i.e. a screener who knows that every one of his/her slides are rescreened as a quality check, and the findings documented, usually screens more carefully.

Rapid rescreening was established at the Ampath laboratory Port Elizabeth in 2000 following the publication of several studies indicating improved sensitivity for the detection of abnormalities in cervical smears. During the study period, 2002 – 2004, as per Table 11 (Chapter 4), 55 583 smears and 48 785 patients with negative results were rapidly rescreened.

A total of 91 smears (0, 19%) were identified as incorrect and selected for full rescreening. Of these, 91 cases were considered inaccurate following pathologist review and resulted in a corrected report or an amended report.

Of these, 29 cases were confirmed as ASC-US; 7 cases were confirmed as ASC-H, 9 cases were confirmed as LSIL and 11 cases as HSIL**.** Thirty-five results were changed in view of minor errors, i.e. wrong recommendations, organisms or viral infections present.

The laboratories that participates in the manual rescreening quality assurance program, normally only screens one of every ten slides – selected at random. This is a very big concern to the author, as selecting a different random collection of slides could lead to a totally opposite result which negates the usefulness of 10% rescreening. With 100% rescreening, personal random choice of slides for rescreening has no influencing on the error rate of the laboratory.

5.3.1 Manual rescreening:

Manual microscopic screening of cervical smears by cytotechnologists continues to be the most widely used and accepted method of identifying irregular cells on cellular abnormalities of the cervix (Cronje, 2004).

At the Ampath laboratory in Port Elizabeth**,** rapid rescreening of all negative, inadequate as well as abnormal smears is the quality control method of choice. Rapid rescreening was easily incorporated into the workflow and it is an effective quality assurance technique resulting in the detection of increased numbers of abnormal smears The calculated concordance rate of the screeners in this laboratory is averaging 99, 81%. This laboratory has an interlaboratory quality assurance program, by comparing the cytology results with that of the histology results. The external quality assurance program of the laboratory has been implemented since 2002. The various cytology laboratories in Port Elizabeth rotate and discuss interesting cases every second Wednesday morning.

At the United Leeds Teaching Hospital in Leeds, rapid rescreening of all negative and inadequate smears is the quality control method of choice. The sensitivity of primary screening of laboratories and individual screeners are major indicators of screening quality and are dependent on the number of false negative smears found by rapid screening their calculation. High sensitivity may indicate good quality primary screening

or poor quality rapid review. The calculated sensitivity in this laboratory ranged from 92-54% for high-grade abnormalities and 75-33% of all grades, revealing a wide range of performance between individual prescreeners. The authorities of this laboratory advise that partial rapid rescreening be considered as the quality control method of choice (Brooke, *et al.*, 2002). The author notes that certain laboratories use prescreeners to divide the smears into two groups – normal and abnormal, before the work is given to the screeners to evaluate. This method is not cost effective in the Ampath laboratory Port Elizabeth (own observation).

At the Laboratory at the Hospital Jeanne de Flandre in France, the author confirms that cytology manual screening is the most appropriate means of screening for cervical cancers and pre-malignant lesions. This laboratory has an interlaboratory quality assurance program, by comparing the cytology results with that of the histology results. They came to the conclusion that the invasive cervical cancers still observed in France can be attributed to the lack of screening experience, lack of management and cytological false negative results. They found that the conditions for optimizing screening are wide coverage of good quality smears, competent cytologists and the appropriate follow-up system of abnormal smears (Leroy & Boman, 2003). The findings of the authors of this laboratory, underlines the 14 contributing factors for absolute quality assurance as discussed in Chapters 1, 2, and 3 (own observation).

The division of Cytology, Boston, Massachusetts, United States of America, wanted to determine the reliability of the false negative rate of cervical cytological screening by rapid rescreening. The test set consisted of 401 cases (311 originally diagnosed as negative, 74 as ASCUS, 14 as LSIL and two as HSIL) and were rapidly (30 seconds) rescreened. Five cytotechnologists with no prior experience in rapid rescreening were tested to evaluate the cases for false negative rating of rapid rescreening and primary screening. Their conclusion was that the false negative rate of rapid rescreening was relatively reproducible though the individual cases identified varied between reviewers. They found that rapid prescreening is the most logistically simple method to determine the true false negative rate of the laboratory (Renshaw, *et al.*, 1999).

The Department of Anatomical Pathology, Queensland Medical laboratory, Brisbane, Australia, had rapid rescreening established in their laboratory in 1995. During this period, 285 841 negative smears (representing 89, 09% of the total workload) were rapidly rescreened and a total of 7 650 (2, 68%) were identified as abnormal or suspicious and selected for full rescreening. They found that rapid rescreening was easily incorporated into their workflow and that rapid rescreening was an effective quality assurance technique resulting in the detection of increased numbers of abnormal smears (Wright, *et al.*, 1999).

A Danish laboratory has found that partial manual rescreening of all negative smears is recommended as the most cost-effective method of internal quality control. The method was tested by 8 cytotechnologists and the average diagnostic sensitivity, assessed upon their answering of 200 test smears (150 negative and 50 positive) was 80%, while specificity was calculated as 95%. Partial rescreening was here-upon used in daily routine. After one year 21 000 smears have been rescreened. Twenty-nine cases of false negatives, 16 with atypia, 8 with koilocytosis, and 5 cases with dysplasia have been detected, which corresponds to an overall false negative rate of 3%. In 5 of the 29 false negative cases with dysplasia, histological follow-up has shown 4 cases of HSIL, and one case of LSIL. Conclusively, partial rescreening of all negative smears implies an improved quality with reduction of the number of false negative specimens (Jensen, *et al.*, 2000).

5.3.2 Automated rescreening:

In the United Kingdom, areas of Europe and United States of America, there is a shortage of qualified cytotechnologists as well as pathologists. These laboratories do not have the staff complement for 100% rescreen and most of them have tried and/or implemented the Automated Papanicolaou smear analysis as a screening tool.

The various laboratories had different findings:

Reviews conducted for the United States Preventive Services Task Force and the Agency for Healthcare Research and quality showed the following: New technologies intended to improve detection of cytological abnormalities included liquid-based, thin-layer cytology (ThinPrep, AutoCyte), computerized rescreening (PAPNET), and algorithm-based computer rescreening (AutoPap). Fourteen % to 33% of the cases failed to detect abnormalities that existed at the time of screening.

Using standard criteria for evaluation of the diagnostic tests, the authors determined that knowledge about the sensitivity, specificity, and predictive values of new technologies, was meagre. Lack of an adequate reference standard was the overwhelming reason that test characteristics could not be properly assessed or compared.

Most publications compared results of screening using the new technology with expert panel review of the cytological specimen. In that case, the test was found not to have independent measures and does nothing to relate the screening test findings to the true status of the cervix, making determination of false-negatives, and thus sensitivity, specificity, and negative predictive value, impossible.

The current evidence for the purposes of guiding decision making about choice of screening tools, proved inadequate to gauge whether new technologies are “better” than conventional cytology (Hartmann, *et al.,* 2001). The fact that these computerized rescreening technologies failed to diagnose 14% to 33% of abnormalities already diagnosed at the time of manual screening, is very alarming and proves to the author that the 100% manual rescreen is much better and much more cost-effective (own observation).

The department of Cellular Pathology, Washington, DC 20306-6000, USA approved several devices that use computerized image analysis to rescreen cervical smears that

have already been examined by cytotechnologists. The objective was to determine the effectiveness and cost of PAPNET-assisted rescreening in identifying cervical abnormalities not identified by manual rescreening.

PAPNET-assisted rescreening of 5478 cervical smears previously identified as "within normal limits" or "benign changes" on both initial and random screening was implemented. The smears were rescreened by PAPNET, followed by re-evaluation of abnormal smears by the consensus panel; consisting of 3 cytotechnologists and 3 pathologists PAPNET screening identified 1614 (29%) slides requiring additional microscopic review. On further review, 448 (8% of total) had possibly abnormal cells.

Ultimately, 11 of these cases were reviewed by the consensus panel for potentially atypical cells. Of these 11 cases, 5 were reclassified as ASC-US and one as AGUS**;** the patient with the diagnosis of AGUS on rescreening was diagnosed as having LSIL on follow-up (O'Leary, *et al.,* 1998).

Five cases of ASC-US was therefore found from 5 478 smears of which the PAPNET originally identified 1 614 slides as requiring additional microscopic review – a pick-up rate of 0,09% compared to the 0,19% pick-up rate of the Ampath laboratory in Port Elizabeth with 100% rescreen (a two-fold increase in pick-up rate).

The author is concerned that only 5 ASC-US cases were identified and no ASC-H, LSIL, HSIL or higher-grade lesions were found. With the 100% manual rescreen in the Ampath laboratory, all grades of intra-epithelial lesions were found and as discussed before, the ASC-H cells can be very few and single-lying and easily interpreted incorrectly (own observation). Does the automated rescreening technology have a problem interpreting the smaller, single-lying abnormal cells?

The Department of Obstetrics and Gynaecology in Los Angeles, California, screened 3 271 patients by simultaneous cervical smears and cervigram, a non-invasive photographic method introduced for screening. The accuracy of each screening test, when positive or

suspicious, was evaluated independently and the results compared with colposcopically directed biopsy results. The cervigram was significantly more sensitive than the cervical smear, whereas the cervical smear was significantly more specific than the cervigram in detecting cervical intra-epithelial neoplasia (Tawa, *et al.*, 1998).

The Medical College of Wisconsin screened 1200 cervical smear slides with negative findings using the PAPNET screening system. Screening with PAPNET identified 8 patients with ASC-US that were not diagnosed on initial screening, yielding a false-negative rate of 0, 7% for ASC-US. No LSIL's or HSIL's were identified. The rate of changed diagnoses in the PAPNET group was similar to the rate in the laboratories standard rescreening of 10% of all smears with negative findings.

The mean turnaround time for a PAPNET screen was 13, 9 days, compared with 3, 9 days for manual review. The conclusion that this laboratory came to, was that for a laboratory with a low percentage of smears with abnormal findings, a quality cytotechnologist and pathologist, and required quality assurance standards in place, PAPNET may not improve the pick-up rate for missed cervical lesions, and may add significantly to the cost and turnaround time of cytological evaluation of smears (Brotzman, *et al.*, 1999).

At The Erasmus University in Rotterdam, The Netherlands, the outcome of having a 10% manual recheck as apposed to PAPNET recheck, was that although PAPNET was found to be time saving as compared to conventional microscopy, the associated reduction in personnel costs is outweighed by the costs of scanning the slides and additional equipment. Using PAPNET instead of conventional microscopy as a primary screening tool will make cervical cancer screening less cost-effective unless the costs of PAPNET are considerably reduced and its sensitivity and/or specificity are considerably improved (Meerding, *et al.*, 2001).

At a laboratory in France, the effectiveness of the PAPNET System was compared with conventional screening. Three thousand ninety-seven negative cervical smears were

rescreened manually and with the PAPNET system. There were two reviews of the PAPNET images. The first by two cytotechnologists with limited exposure to the instrument, and the second, limited to smears with discrepant diagnoses, by an expert in using the system. The results of the smears resreened were compared with available biopsies. On manual resreening of the 3 097 smears, 2 901 (93, 66%) were reported as negative and 170 (5, 49%) as abnormal. On the PAPNET review, 2 938(94, 87%) of the smears were reported as negative and 150 (4, 84%) as abnormal. There were 144 smears with discrepant diagnoses. When compared to the results of the biopsies, PAPNET-assisted rescreening agreed much better with the consensus diagnosis than did manual rescreening. The laboratory has implemented a system where the highest rate of specificity as well as sensitivity is obtained (Bergeron, *et al.*, 2000).

5.4 Conclusion:

5.4.1 Presence or absence of transformation zone component:

The feature of paramount importance in assessing smear quality is that there should be adequate numbers of epithelial cells on the slide, (8000 – 12 000 well visualized squamous cells and 10 squamous metaplastic cells or endocervical cells) with evidence that they are from the appropriate area of the cervix. A smear with an abnormal result is always classified as "satisfactory" even if no transformation zone component is present.

As per Table 11 (Chapter 4) a total of 60 932 smears were scanned

The author is concerned about the 19 243 (31, 58%) patients with negative results and absent transformation component. The concern is thus the lesion-related errors arising from not taking smears with adequate endocervical or transformation zone sampling. At the laboratory concerned, all negative for malignant cells smears with absent endocervical or transformation zone component, except atrophic smears, vault or vaginal smears, has recommendations for the management of these patients. The recommendation for absent component does not require a repeat smear immediately, unless the patient has had high-risk HPV results within 12 months, previous squamous abnormality without 3 subsequent negative cervical smears of which at least one

contained endocervical component, a previous cervical smear with unexplained glandular abnormality, immunosuppression as well as insufficient previous screening. A repeat smear is normally advised after an interval of 4 to 6 weeks to enable the epithelium some time to replenish. A postpartum smear is preferred, but not mandatory for pregnant patients.

5.4.2 Impact of size of coverslips on quality assurance:

Cytology laboratories world-wide vary with the size of coverslips they use, whether they use manual mounting or an automated mounting machine.

At the Ampath laboratory Port Elizabeth, most smears cover the entire non-frosted area of the slide and therefore require the use of the larger coverslip. The workload of the screener is increased by the use of the larger coverslip, but the quality assurance of each smear may not be compromised in any way (own observation).

The author has proven the positive influence of using the larger coverslip for conventional smears, on proper quality assurance and correct diagnoses to which every patient is entitled.

Figure 33 (Chapter 4) illustrates the 2 black markings on the top slide and the 2 black markings on the bottom slide. The rule with using a smaller coverslip is to place this coverslip where the best concentration of cells is on the smear. It is therefore quite obvious that these abnormal cells would have been missed, had a smaller coverslip been used (own observation).

From the year 2000 – 2004, 4, 17% of all abnormal smears had abnormal cells only outside the edge of the smaller coverslip and would therefore have had false-negative results.

This is a total of 223 smears that would have been diagnosed as false negative.

5.4.3 Effect of 100% rescreening of slides on quality assurance:

The author comes to the conclusion that the automated screening technologies have been tested globally, but seems too expensive and its sensitivity and specificity not completely up to standard.

As discussed above, every laboratory has its own perception and findings on the quality control of screening.

In South Africa the cost of automated rescreening is far too high and manual rescreening is depended on. All abnormal smears are rescreened by a 'checker' and then taken to the pathologist (if necessary). It is also the screener's prerogative to hand any smear that they are unsure of, to the checker. The checker also rescreens the smears of all high-risk patients. At the Ampath laboratory in Port Elizabeth, all negative smears are rapid rescreened. Rapid rescreening was established in the laboratory in 2000 following the publication of several studies indicating improved sensitivity for the detection of abnormalities in cervical smears.

A total of 48 785 patients with Negative for malignant cells results were rescreened.

A total of 91 cases (0, 19%) were identified as incorrect and selected for full rescreening.

Manual rescreening has a higher sensitivity and specificity and is much more cost effective.

CHAPTER 6

SUMMARY AND RECOMMENDATIONS

Since cervical cancer is the most common malignancy amongst women in developing countries, the medical profession will have to endeavour to screen a higher rate of women and ensure a 100% quality assurance with every patient treated in order to reduce the unacceptable high incidence rate of cervical carcinoma. The question arises why patients present with advanced cervical disease *ab initio* in countries where cervical screening programs are available. This is regarded as a failure of cervical smear screening.

Therefore we have to concentrate on QA to ensure the quality of cervical pathology in the 41% of patients who present for cervical screening.

As discussed, factors contributing to QA entail a variety of actions which to a lesser or greater extent influences the final diagnosis and follow-up of patients with cervical pathology.

6.1. Taking of smear:

Taking of cervical smears forms the most integral part of quality assurance. With the patient in the lithotomy position, the clinician should visually inspect the cervix and identify the transformation zone. Both a representative endo- and ectocervical sample

should be taken. The percentage of absent transformation zone for normal and abnormal smears are 33, 61% in our laboratory.

6.2. Fixation of smear:

Rapid fixation is essential to ensure the cells are well preserved.

6.3. Marking of slide

The slide should immediately be marked on the frosted end of the slide with a pencil or diamond pen thus preventing slides being mixed up with other patient's forms, resulting in the incorrect slide being screened with the incorrect form.

6.4. Submission of clinical data

Laboratory test results are of limited use in the absence of corresponding clinical information. Clinical information is critical for the technologists, pathologists and clinicians alike with the ultimate goal of optimal patient care.

6.5. Transportation of cervical smears

Timeous fixation is the perfect "transport medium" for a cervical smear and this fixation will keep the cells in perfect condition for a couple of days. No ice packs or additional transport medium is needed.

6.6. Receiving smears in laboratory

Training on the receipt of specimens in the laboratory is regarded as of utmost importance as NO mistakes may be allowed here. The information on the slide is compared with those on the form, and if correct, the slide is assumed to belong to that form and both the slide and the form are allocated a unique laboratory number.

6.7. Staining of slides

Before any diagnosis can be made on any slide, the slide needs to be stained by various stains to enable the screener to interpret the cells with the aid of a microscope. The normal Haematoxylin – Eosin Pap stain is usually used.

6.8 Mounting

Since xylene is a known carcinogen, all mounting of slides should be done under an extractor fan. Entellan of DePex can be used as a mounting medium. The size of the coverslips used is a very important component of quality assurance. By using the 50 x 24 mm size coverslip in our laboratory, 4, 17% (223) of our smears were correctly diagnosed as positive.

6.9 Labeling

Before a slide is labelled, the name and number on the slide as well as the form is compared and the appropriate label is attached to the frosted end of the slide.

6.10 Screening and reporting

Manual microscopic screening of cervical smears by cytotechnologists continues to be the most widely used and accepted method of identifying irregular cells on cellular abnormalities of the cervix.

6.10.1 Liquid-based preparations

Liquid based cytology, or thin layer cytology, is a technique of preparing a monolayer of cells on a glass slide. The technique involves suspending the sample in a solution that is both mucolytic and haemolytic. The sample is concentrated either by centrifugation or by filtration. In the former method a monolayer of cells sediments onto the slide, in the filtration method a monolayer of cells is pressed onto the slide from the filter.

6.10.2 Automation in cytology

Various attempts have been made to automate the microscopic component of cervical screening, which constitutes the main element of data gathering. The microscopic

analysis of smears is not the only area where automation can have an impact. Important advances have already been made in the fields of sample preparation, data storage and manipulation.

Currently, machines are used to assist in staining and mounting slides, and computers are used to record details on patients with their results. The use of computerized data storage has assisted in the analysis of information and provided support to cervical screening programs in the production of printed results, letters and reminders to patients. Automation of cytology screening was tried in South Africa, but found to be too expensive and the specificity and sensitivity not up to standard.

6.11 Typing

Since 2001 The Bethesda system has been implemented in the Ampath laboratory Port Elizabeth and after many typing errors, it was decided to design a worksheet with canned text in order to limit the typing errors. Free text can be used as and when necessary. All diagnoses with their appropriate recommendations as per Bethesda system is incorporated into this canned text system. All screeners and pathologists therefore apply the Bethesda nomenclature and recommendations as per preset canned text.

6.12 Re-check

To reduce the possibility of incorrect assessment of smears by the laboratory, a quality control procedure was introduced in 2000 for all negative and inadequate smears to be rescreened preferably prior to sending out such reports. The process, referred to as “rapid review”, has proven to be helpful in identifying smears wrongly assessed initially as either adequate or inadequate, as well as for finding abnormalities. The 100% recheck definitely aids in the diminishing of the ‘false negative rate.’ Ninety-one (0, 19%) false-negative cases were found with the rapid rescreen.

6.13 Statistics

The statistics of every screener as well as the average error rate of the laboratory is monitored constantly and discussed with the pathologist in charge.

Error rate is:

1. **Concordance rates:** Agreement rate of all screeners and the average of laboratory. This is monitored monthly (99, 81% in Ampath laboratory Port Elizabeth).
2. **ASC-US Rate:** The percentage of ASC-US results should not exceed 5% of the total of smears screened, when a high-risk population is screened. ASC-US should not be used as a collective basket for lack of making a better diagnosis. (Within normal limits in Ampath laboratory Port Elizabeth.)
3. **ASCUS – SIL rate:** The ratio of ASCUS to LSIL + HSIL should not be more than 2-3:1 **(**Within normal limits in Ampath laboratory Port Elizabeth.)

6.14. Comparison to histology specimens

Correspondence with biopsies, etc. received in histology laboratory with a result of previous cervical smear or previous histology report. The cytology and histology result should be compared and even reviewed in certain cases. Should the two results differ considerably, this should be discussed with the submitting health practitioner and recommendations decided upon.

6.15 Recommendation:

Quality assurance is a full-time never-ending task that each medical professional should be completely committed to at all times. Any 'problem' in the laboratory, that can affect the quality of the results of the smears, should be rectified as a matter of urgency. The quality of the cytology results at any laboratory may not be compromised. We should endeavour to implement the QA procedures at all hospitals, clinics and laboratories, to ensure the highest possible degree of quality assurance at all times.

Every person involved with any facet of the medical profession should endeavour to comply to the quality assurance rules completely. In this way, the 41% of women that do go for cervical smears can be assured of the correct diagnosis, the correct follow-up system and the correct treatment. With the follow-up letters in place, the 'lost to follow-up' risk is diminished and the use of the bigger coverslip ensures that as many cells as possible on every smear, is screened and evaluated.

Quality assurance is therefore the key word in every laboratory!

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