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A Practical Manual on Visual Screening for Cervical Neoplasia



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INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

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INTERNATIONAL AGENCY FOR RESEARCH ON CANCER



WORLD HEALTH ORGANIZATION

A Practical Manual on Visual Screening for Cervical Neoplasia

R. Sankaranarayanan, MD
International Agency for Research on Cancer
Lyon, France

Ramani S. Wesley, MD
Regional Cancer Centre,
Thiruvananthapuram, India

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Foreword

Cervical cancer is the second most common cancer among women worldwide, accounting for 452 000 new cases per year. In many developing countries, where an estimated three-fourths of the world burden occurs, it is the most common cancer among women and the most common cause of death among middle-aged women. Despite its public health importance, there are no effective prevention programmes in most developing countries and hence the risk of disease and death from cervical cancer remains largely uncontrolled. Invasive cervical cancers are preceded by a long phase of precancerous lesions that can be detected by screening, and treated effectively by simple treatments which prevent invasive cancer. Cytology-based screening is effective, but beyond the capacity of the health services in many of these countries. Hence, other methods of early detection of cervical neoplasia, particularly those based on visual inspection, are being investigated.

Two simple, low-technology screening tests, namely visual inspection with acetic acid (VIA) and with Lugol's iodine (VILI), which are based on the ability of the trained health-care personnel to detect acetowhite areas or yellow non-iodine uptake areas in the cervical transformation zone, are currently being evaluated in experimental settings as alternatives to cervical cytology. Published results show that VIA has similar sensitivity, but somewhat lower specificity, when compared to quality cytology. For VILI, initial results from several studies in progress indicate that it is another promising screening test.

This manual is intended to help in the training of a range of health-care personnel such as health workers, nurses and physicians to perform VIA and VILI. Draft versions of this manual have been used over the last three years to train health-care personnel in 22 training courses in the context of specific evaluation studies of cervical cancer prevention, supported by the Bill & Melinda Gates Foundation through the Alliance for Cervical Cancer Prevention (ACCP) in Angola, Burkina Faso, Congo, Guinea, India, Mali, Mauritania, Nepal, Laos, Senegal and Tanzania. Feedback from the participants in these courses and test providers has been particularly useful in revising draft versions of the manual, which is expected to fulfil the long-felt need for a simple learning resource to provide proper and adequate training of health-care personnel and to ensure skilled practice of VIA and VILI in experimental and clinical detection settings.

P. Kleihues MD
Director, IARC

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Chapter 1

Anatomical and pathological basis of visual inspection with acetic acid (VIA) and with Lugol's iodine (VILI)

Introduction

Naked-eye visual inspection of the uterine cervix, after application of 5% acetic acid (VIA) and/or of Lugol's iodine (VILI), provides simple tests for the early detection of cervical precancerous lesions and early invasive cancer. VILI is similar to the Schiller's iodine test, which was used for early detection of cervical neoplasia in the third and fourth decades of the 20th century, but discontinued after the advent of cervical cytology testing. The potential difficulties in implementing cervical cytology-based screening in low-resource settings have prompted the investigation of the accuracy of alternative low-technology tests such as VIA and VILI in the early detection of cervical neoplasia.

The results of VIA and VILI are immediately available and do not require any laboratory support. The categorization of the results of VIA or VILI depends upon the colour changes observed on the cervix. A clear understanding of the anatomy, physiology and pathology of the cervix is absolutely essential to understand the basis and to interpret the outcome of screening using VIA and VILI. The objective of this manual is to help a range of health-care providers such as doctors, nurses, midwives and health workers to acquire

the skills and competence in administering and reporting the results of these tests by describing their basis and practice.

Gross anatomy of the uterine cervix

The cervix, constituting the lower portion of the uterus, is cylindrical or conical in shape, and measures 3-4 cm in length and 2.5-3.5 cm in diameter. It varies in size and shape depending on the age, parity and hormonal status of the woman. The lower half of the cervix, called portio vaginalis, protrudes into the vagina through its anterior wall, and the upper half, called the supravaginal portion, remains above the vagina ([Figure 1.1](#)). The cervix opens into the vagina through the external os. The supravaginal portion meets the body of the uterus at the internal os. In parous women, the cervix is bulky and the external os appears as a wide, gaping, transverse slit. In nulliparous women, the external os resembles a small circular (pinhole) opening.

The portion of the cervix that is exterior to the external os is called the ectocervix, which is readily visible during speculum examination. The portion above the external os is called the endocervix. The endocervical canal, which traverses the endocervix, connects

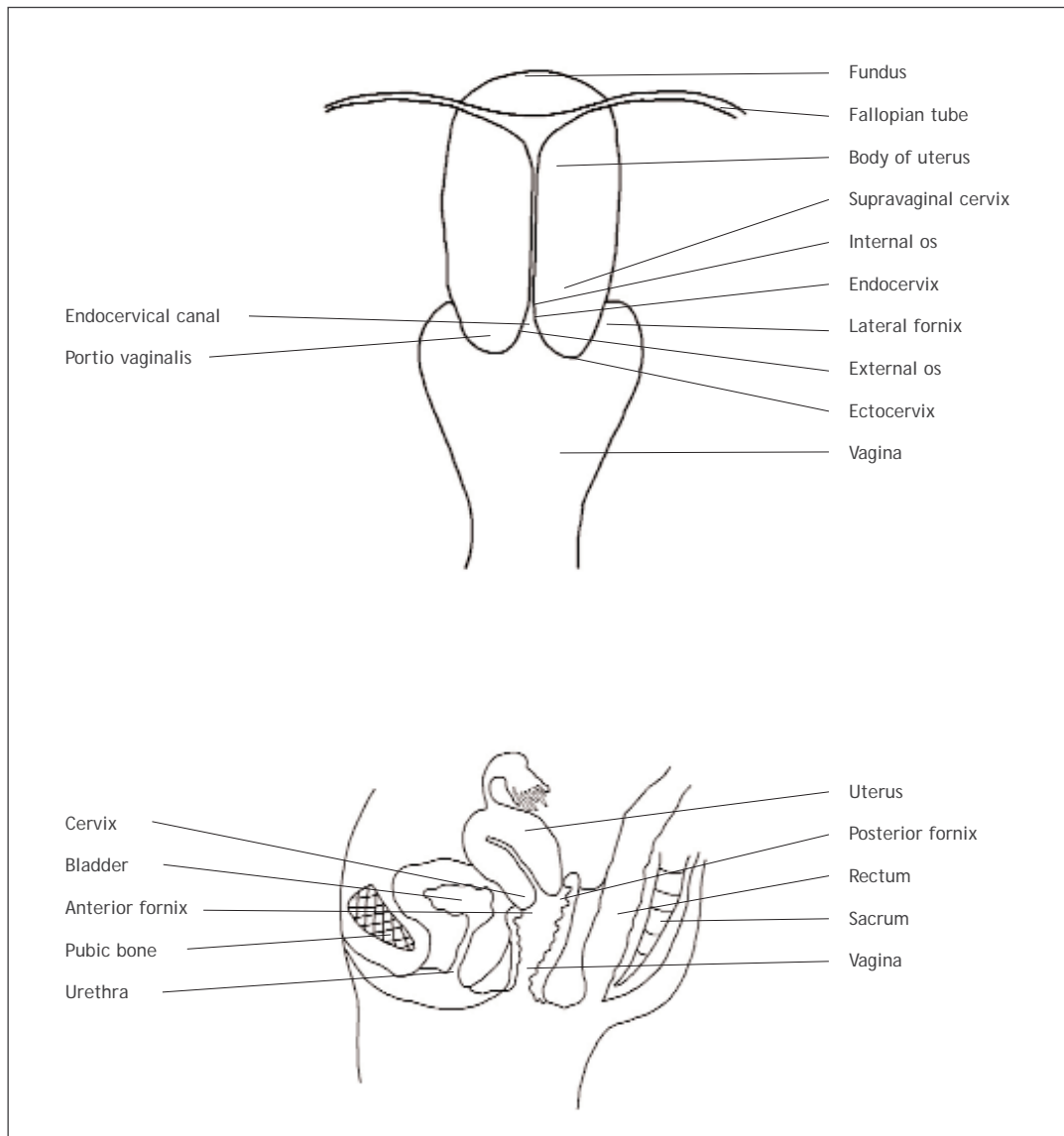


FIGURE 1.1: Gross anatomy of the uterine cervix

the uterine cavity with the vagina and extends from the internal to the external os. The portion of the upper vaginal cavity that surrounds the portio vaginalis is called the fornix.

The stroma of the cervix is composed of dense, fibro-muscular tissue traversed by the vascular, lymphatic and nerve

supplies to the cervix. The arteries of the cervix, derived from internal iliac arteries through the cervical and vaginal branches of the uterine arteries, descend in the lateral aspects of the cervix at 3 and 9 o'clock positions. The veins run parallel to the arteries and drain into the hypogastric venous plexus. The lymphatic

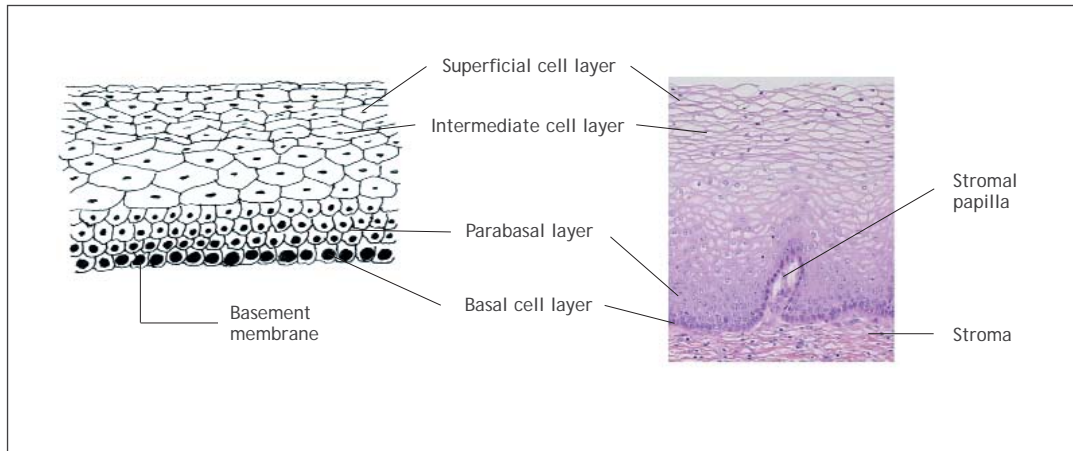


FIGURE 1.2: Stratified squamous epithelium (x20)

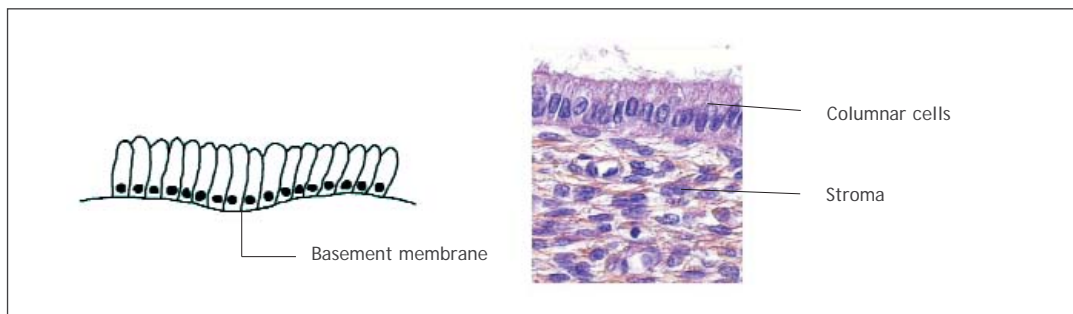


FIGURE 1.3: Columnar epithelium (x40)

vessels from the cervix drain into the common, internal and external iliac nodes, obturator and the parametrial nodes. The nerve supply is derived from the hypogastric plexus. The endocervix has extensive sensory nerve endings, while there are very few in the ectocervix. Hence, procedures such as biopsy and cryotherapy are well tolerated in most women, without local anaesthesia. Since sympathetic and parasympathetic fibres are also abundant in the endocervix, manipulation of the endocervix may stimulate these nerve endings, occasionally leading to giddiness

or fainting attacks.

Microscopic anatomy *Squamous epithelium*

The cervix is covered by two types of epithelium, stratified squamous epithelium and columnar epithelium, which meet at the squamocolumnar junction. A large area of ectocervix is covered by the stratified, non-keratinizing, glycogen-containing squamous epithelium. It is opaque, has multiple (15-20) layers of cells (Figure 1.2) and appears pale pink in colour on visual examination. It consists of a single layer of round basal cells with a large dark-staining

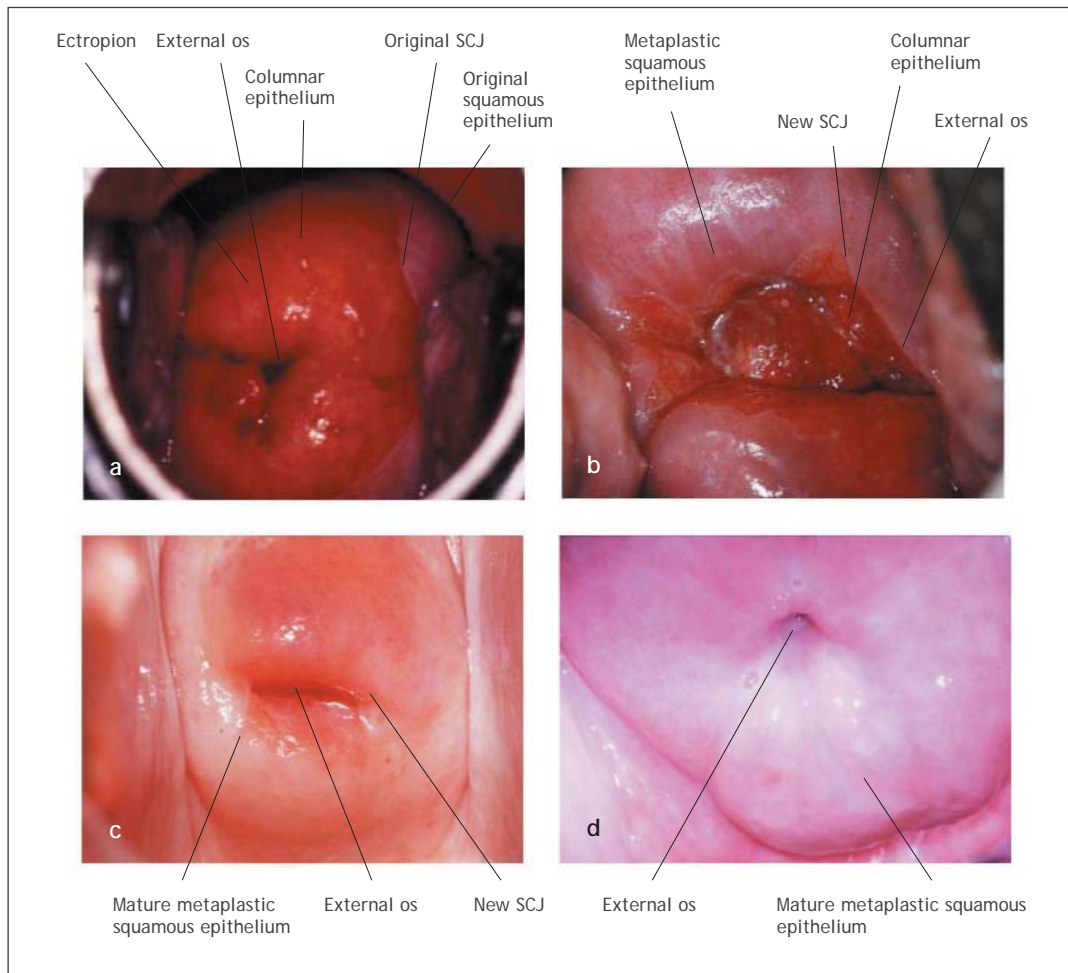


FIGURE 1.4: Location of squamocolumnar junction (SCJ)

- (a) Original squamocolumnar junction (SCJ) in a young woman in the early reproductive age group. The SCJ is located far away from the external os. Note the presence of everted columnar epithelium occupying a large portion of the ectocervix producing ectropion.
- (b) The new SCJ has moved much closer to the external os in a woman in her 30s. The SCJ is visible as a distinct white line after the application of 5% acetic acid due to the presence of immature squamous metaplastic epithelium adjacent to the new SCJ.
- (c) The new SCJ is at the external os in a perimenopausal woman.
- (d) The new SCJ is not visible and has receded into the endocervix in a postmenopausal woman. Mature metaplastic squamous epithelium occupies most of the ectocervix.

nucleus and little cytoplasm at the basement membrane. The basement membrane separates the epithelium from the underlying stroma. The basal cells

divide and differentiate to form the parabasal, intermediate and superficial layers. From the basal to the superficial layer, the cells undergo an increase in

cytoplasm and a reduction of nuclear size. The intermediate and superficial layer cells contain abundant glycogen in their cytoplasm. Since iodine readily stains with glycogen, application of Lugol's iodine on the squamous epithelium will result in mahogany brown or black coloration. In postmenopausal women, the cells in the squamous epithelium do not mature beyond the parabasal layer, and do not accumulate as multiple layers of intermediate and superficial cells. Consequently, the squamous epithelium becomes thin and atrophic. Thus, it appears pale and brittle, with sub-epithelial petechiae, as it is easily prone to trauma.

Columnar epithelium

The endocervical canal is lined by the columnar epithelium (sometimes referred to as glandular epithelium), composed of a single layer of tall cells with dark-staining nuclei (Figure 1.3). On visual examination, it appears as a grainy, strikingly reddish area because the thin single cell layer allows the coloration of the underlying stroma to be seen more easily. It forms several invaginations into the substance of the cervical stroma, resulting in the formation of endocervical crypts (sometimes referred to as endocervical glands). The columnar cells secrete the mucus that lubricates the cervix and vagina. At its upper limit, it merges with the endometrial epithelium in the body of uterus, and at its lower limit, it meets with the squamous epithelium at the squamocolumnar junction. A localized proliferation of the columnar epithelium in the form of a polyp may occasionally be visible as a reddish mass protruding from the external os (Figure 1.5). As columnar epithelium does not produce glycogen, it

does not change colour after the application of Lugol's iodine, or remains slightly discoloured with a thin film of iodine solution.

Squamocolumnar junction

The squamocolumnar junction (Figure 1.4) appears as a sharp line. The location of the squamocolumnar junction in relation to the external os varies, depending upon factors such as age, hormonal status, birth trauma and certain physiological conditions such as pregnancy (Figure 1.4). During childhood and perimenarche, it is located at, or very close to, the external os. After puberty and during the reproductive period, the female genital organs grow under the influence of estrogen. Thus, the cervix enlarges and the endocervical canal elongates. This leads to the eversion of the columnar epithelium onto the ectocervix, particularly on the anterior and posterior lips, resulting in ectropion or ectopy. Thus, the squamocolumnar junction is located in the ectocervix, far away from the external os during the reproductive years and pregnancy (Figure 1.4a). On visual inspection, ectropion is seen as a strikingly reddish ectocervix (Figure 1.4a).

The buffer action of the mucus covering the columnar cells is interfered with when the everted columnar epithelium is exposed to the acidic vaginal environment. This leads to the destruction and eventual replacement of the columnar epithelium by the newly formed metaplastic squamous epithelium. Metaplasia refers to the change or replacement of one type of epithelium by another. As a woman passes through her reproductive life to the perimenopausal age group, the location of the squamocolumnar junction progressively starts moving on the ectocervix towards the external os (Figures 1.4b and c). Thus, it is located at variable

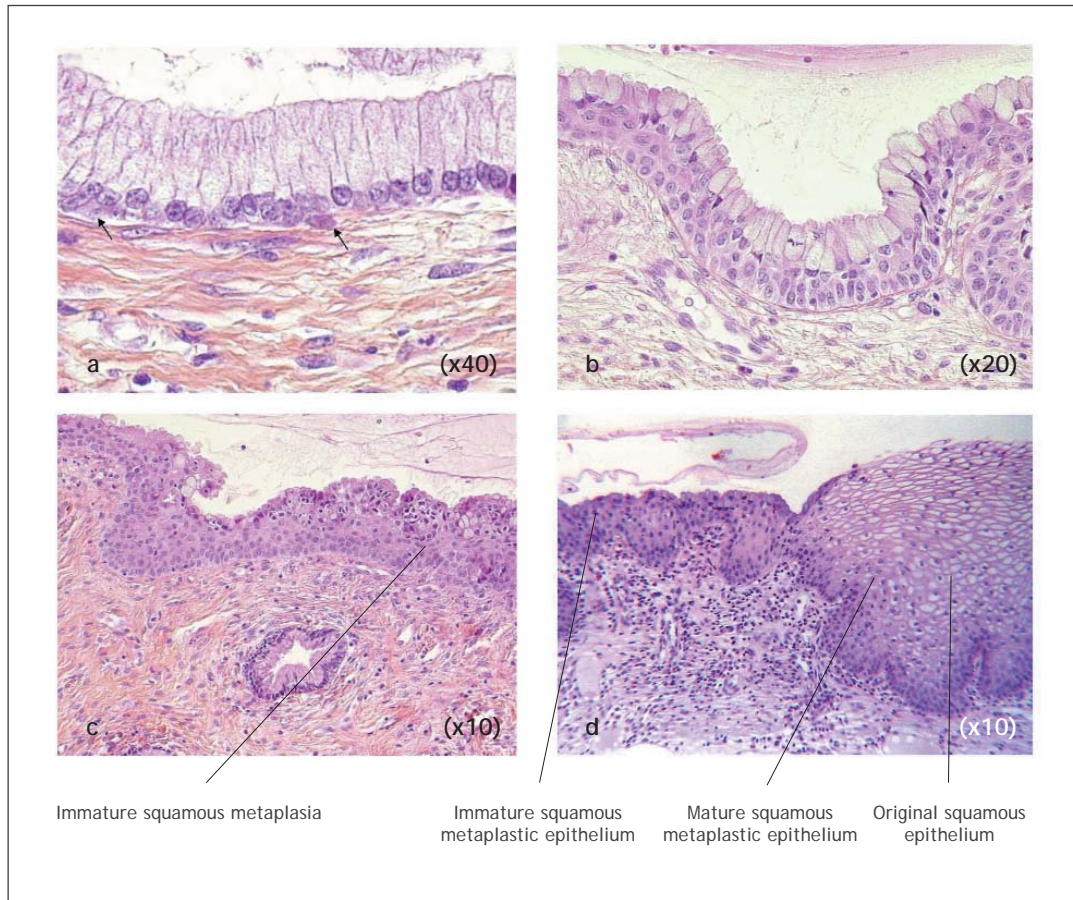


FIGURE 1.5: Development of squamous metaplastic epithelium

- (a) The arrows indicate the appearance of the subcolumnar reserve cells.
- (b) The reserve cells proliferate to form two layers of reserve cell hyperplasia beneath the overlying layer of columnar epithelium.
- (c) The reserve cells further proliferate and differentiate to form immature squamous metaplastic epithelium. There is no evidence of glycogen production.
- (d) Mature squamous metaplastic epithelium is indistinguishable from the original squamous epithelium for all practical purposes.

distances from the external os, as a result of the progressive formation of the new metaplastic squamous epithelium in the exposed areas of the columnar epithelium in the ectocervix. From the perimenopausal period and after the onset of menopause, the cervix shrinks, due to the lack of estrogen, and, consequently, the movement

of the squamocolumnar junction towards the external os and into the endocervical canal is further accelerated (Figure 1.4c). In postmenopausal women, the squamocolumnar junction is located in the endocervical canal and, hence, often cannot be seen on visual examination (Figure 1.4d).

Squamous metaplasia

The earliest event in squamous metaplasia is the appearance of small, round, sub-columnar cells in the exposed areas of the columnar epithelium, called reserve cells (Figure 1.5a). These reserve cells proliferate (Figure 1.5b) and differentiate to form a thin, non-stratified, multicellular epithelium called immature squamous epithelium (Figure 1.5c). The cells in the immature squamous metaplastic epithelium do not produce glycogen and, hence, do not stain brown or black with Lugol's iodine solution. Numerous foci of immature squamous metaplasia may arise at the same time.

Further development of the newly formed immature metaplastic epithelium may take either one of two directions (Figure 1.6). In the vast majority of women, it develops into a mature, stratified, glycogen-producing, squamous metaplastic epithelium, which is similar to the squamous epithelium found on the ectocervix, for all practical purposes (Figure 1.5d). Thus, it stains brown or black after the application of Lugol's iodine. Several cysts, called nabothian cysts, may be observed in the mature metaplastic squamous epithelium (Figure 2.3). These are retention cysts that develop as a result of the occlusion of the crypt openings in the trapped columnar epithelium by the overlying metaplastic squamous epithelium. The buried columnar epithelium in the cysts may continue to secrete mucus, which eventually distends the cysts. The entrapped mucus gives an ivory-white hue to the cyst on visual examination.

In a very small minority of women, the immature squamous metaplasia may turn into a dysplastic epithelium (an altered

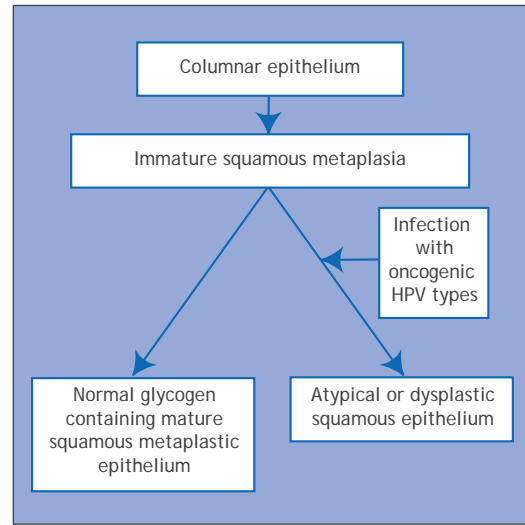


FIGURE 1.6: A schematic diagram of further maturation of immature squamous metaplasia.

epithelium showing precancerous cellular changes), due to infection with certain human papillomavirus (HPV) types (Figure 1.6).

Transformation zone

The transformation zone is the area of the cervix where the columnar epithelium has been replaced and/or is being replaced by the metaplastic squamous epithelium. With the naked eye, one can identify the inner border of the transformation zone by tracing the squamocolumnar junction and the outer border by locating the distal most nabothian cysts (if present) or crypt openings (usually visible under magnification). In premenopausal women, the transformation zone is primarily located on the ectocervix. After menopause, and through old age, the cervix shrinks with the decreasing levels of oestrogens. Consequently, the transformation zone may move partially,

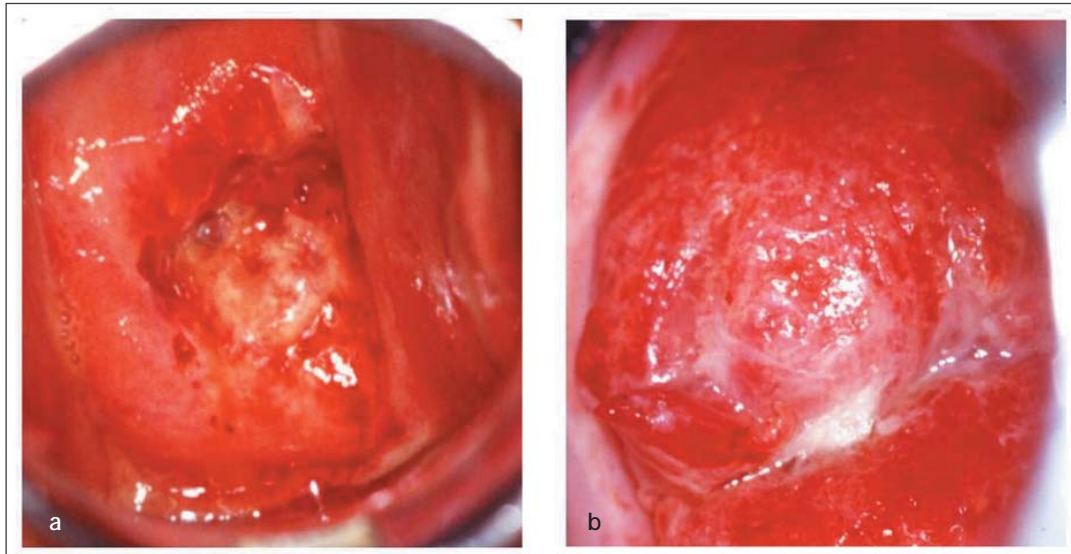


FIGURE 1.7:

- (a) An inflamed cervix, with ulceration, bleeding, necrosis, greenish-yellow discharge and inflammatory exudate.
- (b) A reddish angry-looking, inflamed cervix with loss of the villi in the columnar epithelium and covered with inflammatory exudate.

and later fully, into the endocervical canal. Almost all cervical neoplasia occurs in this zone, close to the squamocolumnar junction.

Inflammation of the uterine cervix (Figure 1.7)

The most common pathological condition affecting a woman's cervix is inflammation. This is caused mostly by infection (usually polymicrobial) and, less commonly, by foreign bodies (retained tampon, etc.), trauma and chemical irritants such as gels and creams. The infectious agents causing inflammation in the cervix include: *Trichomonas vaginalis*; *Candida albicans*; overgrowth of anaerobic bacteria such as *Gardnerella vaginalis*, *G. mobiluncus* and peptostreptococcus; other bacterial infections such as *Haemophilus ducreyi*, *Neisseria gonorrhoeae*, *Chlamydia*

trachomatis, *Escherichia coli*, streptococci, and staphylococci; and viral infections such as Herpes simplex.

Columnar epithelium is more prone to infection than squamous epithelium. We use the term cervicitis in this manual to denote all cervicovaginal inflammatory conditions. Clinically, cervicitis may be associated with symptoms such as excessive discharge, itching of the vulva and vagina, pain and a burning sensation during sexual intercourse and lower abdominal pain. Clinical signs include excessive, coloured (greyish, greyish-white, curdy-white (in the case of candidal infection), yellow or greenish-yellow), malodorous or non-odorous, frothy or non-frothy secretions, tender, reddish cervix with or without vesicles, ulcerations and/or fibrosis; the columnar epithelium may look flattened; and there may be

excoriation marks on the vulva, vulval erythema and oedema, vagina and inner thigh and perineum. Microscopically, cervicitis is characterized by cellular debris and excessive secretions covering the epithelium, swollen and inflamed cells, desquamation of the glycogen-containing superficial and intermediate cells, epithelial denudation, superficial or deep ulceration and congestion of the underlying cervical stroma. Chronic inflammation results in recurrent ulceration and may lead to healing by fibrosis.

A diagnosis of cervicitis can be made based on the clinical features. On visual examination, cervicitis due to non-candidial infection may be characterized by vulval erythema and oedema, excoriation marks in the vulva and vagina and a reddish, tender cervix with malodorous, greenish yellow or greyish-white mucopurulent discharge, with or without ulceration. In the case of gonococcal cervicitis, painful urethral discharge is also observed. Candidial cervicitis is characterized by vulval oedema and erythema, excoriation, and thick, curdy-white, non-odorous discharge. Herpes infection is associated with the presence of vesicles and ulcers in the external genitalia, vagina and the cervix,

as well as cervical tenderness. Women with non-candidial cervicitis may be treated with a combination of metronidazole 400 mg plus doxycycline 100 mg orally, two times a day for seven days. Those with candidial cervicitis may be treated with clotrimazole or miconazole 200 mg intravaginally, daily for three days.

Cervical neoplasia

Invasive cervical cancers are usually preceded by a long phase of preinvasive disease, characterized microscopically as a spectrum of precursor lesions progressing from cellular atypia to various grades of cervical intraepithelial neoplasia (CIN) before progression to invasive carcinoma. Epidemiological studies have identified a number of risk factors that contribute to the development of CIN and cervical cancer. These include infection with certain types of human papillomavirus (HPV), sexual intercourse at an early age, multiple sexual partners, multiparity, long-term oral contraceptive use, tobacco smoking, low socioeconomic status, infection with *Chlamydia trachomatis*, micronutrient deficiency and a diet deficient in vegetables and fruits. HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 are strongly associated with

Table 1: Correlation between CIN, dysplasia and the Bethesda

CIN 1	CIN 2	CIN 3
Mild dysplasia	Moderate dysplasia	Severe dysplasia Carcinoma <i>in situ</i>
Low-grade squamous intraepithelial lesion (LSIL)	High-grade squamous intraepithelial lesion (HSIL)	High-grade squamous intraepithelial lesion (HSIL)

CIN and invasive cancer. Persistent infection with one or more of the above HPV types is considered to be a necessary cause for cervical neoplasia.

Infection with one or more of the oncogenic HPV types may result in the integration of the viral genome into the host cellular genome resulting in the formation of cervical neoplastic cells, the proliferation of which leads to various grades of CIN (synonyms: dysplasia or squamous intraepithelial lesions (SIL)), which may progress to invasive cervical cancer. The correlation between the CIN terminology, used in this manual, and other terminologies is given in Table 1.

Cervical intraepithelial neoplasia

There are no specific symptoms or visible signs associated with CIN. However, the presence of CIN may be suspected by the naked-eye detection of well defined, acetowhite areas in the transformation zone, close to or abutting the squamocolumnar junction, after the application of 3-5% acetic acid or of well defined mustard or saffron yellow iodine non-uptake areas in the transformation zone

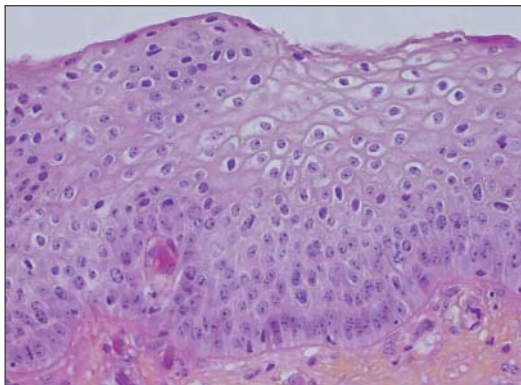


FIGURE 1.8:
Histology of CIN 1: The dysplastic cells are confined to the lower third of the epithelium x20.

after application of Lugol's iodine solution.

The final diagnosis of CIN is established

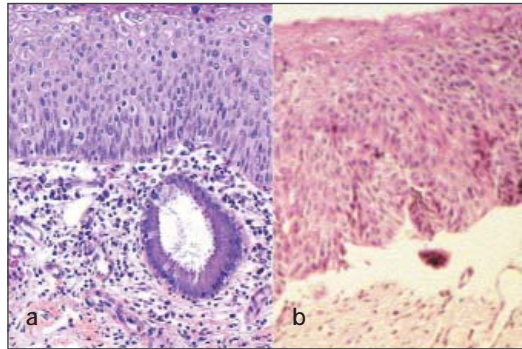


FIGURE 1.9:
Histology of CIN 2: Atypical cells are found mostly in the lower two-thirds of the epithelium x10.

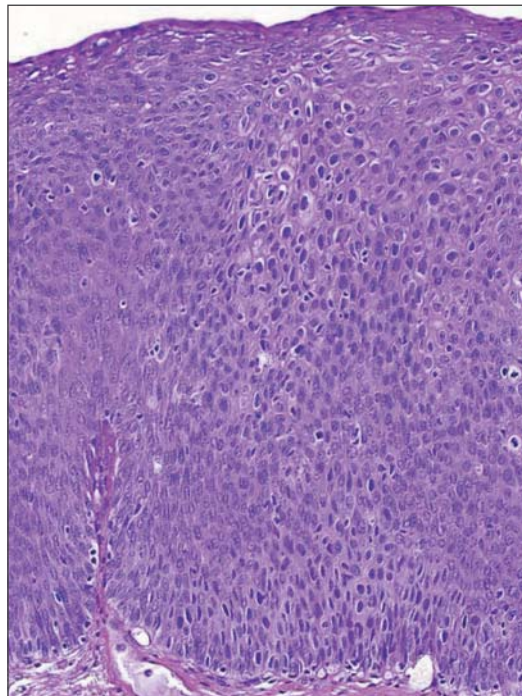


FIGURE 1.10:
Histology of CIN 3: Dysplastic cells are distributed in the full thickness of the epithelium with loss of polarity of cells x20.

by histopathological examination of tissue specimens from the cervix. The undifferentiated cells in CIN are characterized by enlarged nuclei, increased intensity of nuclear staining, nuclear polymorphism and variation in nuclear size, and a decreased amount of cytoplasm, resulting in a higher nuclear cytoplasmic ratio. The proportion of the thickness of the epithelium showing undifferentiated cells is used for grading CIN. In CIN 1 the undifferentiated cells are confined to the deeper layers (lower third) of the epithelium (Figure 1.8). Mitotic figures are present, but not very numerous. CIN 2 is characterized by dysplastic cellular changes mostly restricted to the lower half or the lower two-thirds of the epithelium, with more marked nuclear abnormalities than in CIN 1 (Figure 1.9). Mitotic figures may be seen throughout the lower half of the epithelium. In CIN 3, differentiation and stratification may be totally absent or present only in the superficial quarter of the epithelium with numerous mitotic figures (Figure 1.10). Nuclear abnormalities extend throughout the thickness of the epithelium. Many mitotic figures have abnormal forms.

It is well established that most CIN 1 lesions are transient; most of them regress to normal, within relatively short periods, or do not progress to higher grades. High-grade CIN (CIN 2-3), on the other hand, carries a much higher probability of progressing to invasive cancer, although a large proportion of such lesions also regress or persist. It is assumed that the mean interval for progression of cervical precursors to invasive cancer may be as long as 10 to 20 years.

Women with CIN are treated with cryotherapy, loop electrosurgical excision procedure (LEEP) or cold-knife conization.

Women with CIN 1 may be advised to undergo immediate treatment (e.g., in situations where follow-up of women cannot be assured) or treated later if two follow-up visits at six or nine months apart reveal persistent or progressive disease.

The precursor lesion that arises from the columnar epithelium is referred to as adenocarcinoma *in situ* (AIS). In AIS, normal columnar epithelium is replaced by abnormal epithelium showing abnormal, irregularly arranged cells with increased size of cells and nuclei, nuclear hyperchromasia, mitotic activity, reduction of cytoplasmic mucin expression and cellular stratification.

Invasive cancer

In very early phases of invasion, cervical cancer may not be associated with obvious symptoms and signs, and, therefore, is known as preclinical invasive cancer. Women with moderately advanced or advanced invasive cervical cancer often present with one or more of the following symptoms: intermenstrual bleeding, postcoital bleeding, excessive seropurulent discharge, recurrent cystitis, backache, lower abdominal pain, oedema of the lower extremities, obstructive uropathy, bowel obstruction, breathlessness due to severe anaemia and cachexia.

As the stromal invasion progresses, the disease becomes clinically obvious, showing several growth patterns, which are visible on speculum examination. Early lesions may present as a rough, reddish, granular area that bleeds on touch (Figure 1.11). More advanced cancers may present as a proliferating, bulging, mushroom- or cauliflower-like growth with bleeding and foul-smelling discharge (Figure 1.12). Occasionally they may present without much surface



FIGURE 1.11:
Early invasive cervical cancer: note the irregular, granular, nodular surface with bleeding on touch.

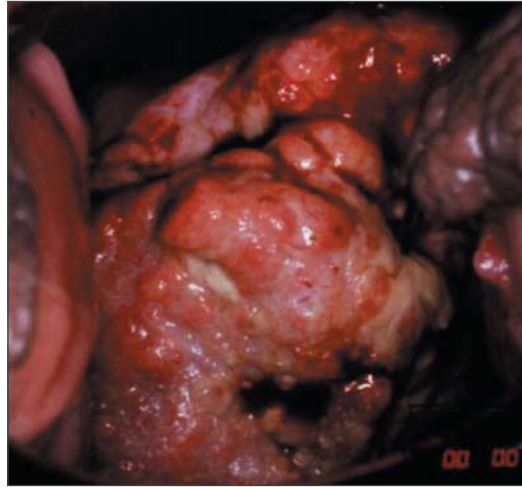


FIGURE 1.12:
Advanced invasive cervical cancer: note the bulging, cauliflower-like, ulceroproliferative growth with bleeding and necrosis.

growth, resulting in a grossly enlarged, irregular cervix with a rough, granular surface.

As the invasion continues further, it may involve the vagina, parametrium, pelvic sidewall, bladder and rectum. Compression of the ureter, due to advanced local disease, causes ureteral obstruction, which results in hydronephrosis and, ultimately, renal failure. Regional lymph node metastasis occurs along with local invasion. Metastatic cancer in para-aortic nodes may extend through the node capsule and directly invade the vertebrae and nerve roots causing back pain. Direct invasion of the branches of the sciatic nerve roots causes low back pain and leg aches, and encroachment of the pelvic wall veins and lymphatics causes oedema of the lower limbs. Distant metastases occur late in the disease, usually involving para-aortic nodes, lungs, liver, bone and other structures.

Histologically, approximately 90-95% of invasive cervical cancers in developing

countries are squamous cell cancers (Figure 1.13) and 2-8% are adenocarcinomas (Figure 1.14). It is obligatory that all invasive cancers be clinically staged. The most widely used staging system for cervical cancer was developed by the International Federation of Gynecology and Obstetrics (FIGO) (see Appendix 1). This is primarily a clinical staging system based on tumour size and extension of the disease in the pelvis. The extent of growth of cancer is assessed clinically, as well as by various investigations, to categorize the disease stages I through IV. Stage I represents growth localized on the cervix, while stage IV represents the growth phase in which the cancer has spread to distant organs by metastasis.

Women with early invasive cancers (stages I and II A) may be treated with radical surgery and/or radiotherapy. Those with stage IIB and III cancers may be treated with radiotherapy with or without cisplatin-based chemotherapy. Women

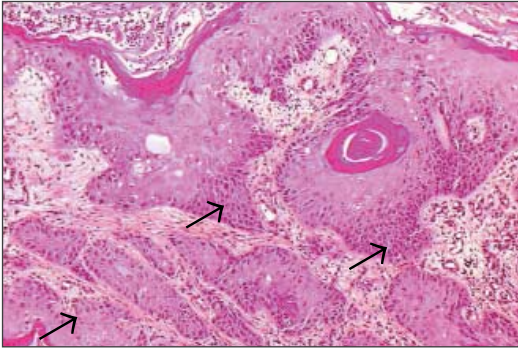


FIGURE 1.13:
Histology – Keratinizing well differentiated invasive squamous cell carcinoma. Note the stroma is infiltrated by sheets of malignant cells x10.

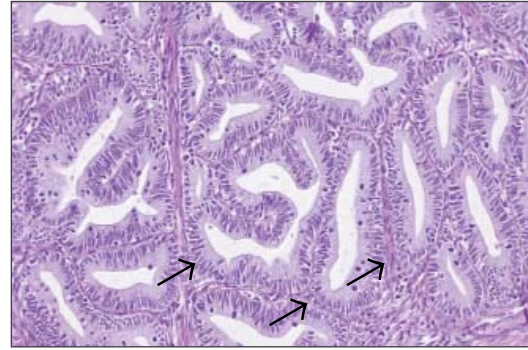


FIGURE 1.14:
Histology – Well differentiated invasive adenocarcinoma. Note the malignant cells lining the cervical crypts x20.

with stage IV cancers are usually treated with palliative radiotherapy and/or chemotherapy and with symptomatic measures.

Other conditions

Leukoplakia (hyperkeratosis) is a well demarcated white area on the cervix (before the application of acetic acid), due to keratosis, visible to the naked eye. Usually leukoplakia is idiopathic, but it may also be caused by chronic foreign body irritation, HPV infection, or squamous neoplasia. *Condylomata* or genital warts are often multiple, exophytic lesions that are usually found on the cervix, and occasionally in the vagina and on the vulva, caused by infection with certain HPV types such as 6 and 11. They may also present as a diffuse, greyish-white lesion involving areas of the cervix and vagina. Condylomata may be obvious to the naked eye (before the application of acetic acid).

Pathophysiological basis of VIA

Application of 5% acetic acid is believed to cause a reversible coagulation, or

precipitation of the cellular proteins. It also causes swelling of the epithelial tissue, columnar and any abnormal squamous epithelial areas in particular, dehydration of the cells, and it helps in coagulating and clearing the mucous secretions on the cervix. The normal squamous epithelium appears pink and the columnar epithelium red, due to the reflection of light from the underlying stroma, which is rich in blood vessels. If the epithelium contains a lot of cellular proteins, acetic acid coagulates these proteins, which may obliterate the colour of the stroma. The resulting acetowhitening is seen distinctly as compared with the normal pinkish colour of the surrounding normal squamous epithelium of the cervix, an effect that is commonly visible to the naked eye. Thus, the effect of acetic acid depends upon the amount of cellular proteins present in the epithelium. Areas of increased nuclear activity and DNA content exhibit the most dramatic white colour change.

When acetic acid is applied to normal squamous epithelium, little coagulation

occurs in the superficial cell layer, as this is sparsely nucleated. Although the deeper cells contain more nuclear protein, the acetic acid may not penetrate sufficiently and, hence, the resulting precipitation is not sufficient to obliterate the colour of the underlying stroma. Areas of CIN and invasive cancer undergo maximal coagulation due to their higher content of nuclear protein (in view of the large number of undifferentiated cells contained in the epithelium) and prevent light from passing through the epithelium. As a result, the sub-epithelial vessel pattern is obliterated and the epithelium appears densely white. In CIN, acetowhitening is restricted to the transformation zone close to the squamocolumnar junction, while in cancer it often involves the entire cervix.

The acetowhite appearance is not unique to CIN and early cancer. It is also seen in other conditions when increased nuclear protein is present, as in immature squamous metaplasia, in healing and regenerating epithelium (associated with inflammation), leukoplakia (hyperkeratosis) and condyloma. While the acetowhite epithelium associated with CIN and early invasive cancer is more dense, thick and opaque with well demarcated margins from the surrounding normal epithelium, the acetowhitening associated with immature squamous metaplasia, inflammation and regenerating epithelium is less pale, thin, often translucent, and patchy with ill-defined margins. Acetowhitening due to inflammation and healing is usually distributed widely in the cervix, not restricted to the transformation zone and may quickly disappear (within a minute). Leukoplakia and condylomata appear intensely greyish-white after the application of acetic acid.

The acetic acid effect reverses much more slowly in CIN lesions and in early preclinical invasive cancer than in immature squamous metaplasia and inflammation. It appears rapidly and may last for 3-5 minutes in the case of CIN 2-3 and invasive cancer.

Pathophysiological basis of VILI

Squamous metaplastic epithelium is glycogenated, whereas CIN and invasive cancer cells contain little or no glycogen. Columnar epithelium does not contain glycogen. Immature squamous metaplastic epithelium usually lacks glycogen or, occasionally, may be partially glycogenated. Iodine is glycophilic and hence the application of iodine solution results in uptake of iodine in glycogen-containing epithelium. Therefore, the normal glycogen-containing squamous epithelium stains mahogany brown or black after application of iodine. Columnar epithelium does not take up iodine and remains unstained, but may look slightly discoloured due to a thin film of iodine solution; areas of immature squamous metaplastic epithelium may remain unstained with iodine or may be only partially stained. If there is shedding (or erosion) of superficial and intermediate cell layers associated with inflammatory conditions of the squamous epithelium, these areas do not stain with iodine and remain distinctly colourless in a surrounding black or brown background. Areas of CIN and invasive cancer do not take up iodine (as they lack glycogen) and appear as thick mustard-yellow or saffron coloured areas. Areas with leukoplakia (hyperkeratosis) do not stain with iodine either, and condylomata may not, or occasionally may only partially, stain with iodine.

Chapter 2

Testing and reporting the results of visual inspection with 5% acetic acid (VIA)

Instruments and materials required:

- Examination table with knee crutches or leg rests or stirrups;
- Good light source (preferably a bright halogen lamp that can be easily directed at the cervix or a bright halogen torch light);
- Sterile bivalved speculum: Cusco's, Grave's or Collin's;
- Pair of gloves;
- Cotton swabs, cotton-tipped buds, gauze;
- Ring forceps, pickup forceps;
- 5% freshly prepared acetic acid or vinegar (check the strength of acetic acid in vinegar);
- A steel/plastic container with 0.5% chlorine solution in which to immerse the gloves;
- A plastic bucket or container with 0.5% chlorine solution to decontaminate instruments;
- A plastic bucket with a polythene bag to dispose of contaminated swabs and other waste items.

Preparation of 5% dilute acetic acid

5% acetic acid is prepared by adding 5 ml of glacial acetic acid into 95 ml of distilled water.

If vinegar bought from a store is used, check the strength to ensure that it is 5%.

Test provider skills

The test provider must have a good knowledge of the anatomy, physiology and pathology of the cervix in relation to its visual examination. He/she should know the clinical features of benign conditions, inflammation, precancerous lesions and invasive cancer of the cervix.

Procedure

Women coming for testing should have the screening procedure explained to them in detail. Written informed consent should be obtained before screening. An example of a written informed consent form is given in Appendix 2. Relevant obstetric and gynaecological history should be obtained and recorded with the help of a form for this purpose (Appendix 3). The woman should be reassured that the procedure is painless, and every effort should be made to ensure that she is fully relaxed and remains at ease during testing.

The woman is invited to lie down in a modified lithotomy position on a couch with leg rests or knee crutches or stirrups. After proper positioning of the woman, observe if there is any vaginal discharge. Observe the external genitalia and perineal region for any signs of excoriations, oedema, vesicles, papules, sores, ulceration and warts.

Look for any swelling in the inguinal/femoral region.

Afterwards, gently introduce a sterile vaginal speculum which has been immersed in warm water and open the blades of the speculum to view the cervix. Adjust the light source so that there is adequate light in the vagina and on the cervix. As the speculum is gently opened and the lips are fixed, the cervix comes into view. Observe the size and shape of the cervix.

Identify the external os, columnar epithelium (red in colour), squamous epithelium (pink) and the squamocolumnar junction. Proceed to identify the transformation zone, the upper limit of which is formed by the squamocolumnar junction. Remember that cervical neoplasias occur in the transformation zone nearest to the squamocolumnar junction.

Look for ectropion, cervical polyp, nabothian cysts, healed laceration of the cervical lips, leukoplakia, condylomata and signs of cervicitis. You may note that in postmenopausal women, the cervix appears pale and brittle, due to thinning and atrophy of the squamous epithelium. Assess the characteristics of discharge in terms of quantity, colour, odour and thickness. Thread-like, thin mucinous discharge from the external os indicates ovulation. If heavy blood flow through the external os is observed in women during menstruation, they may be subjected to VIA after 5-15 days.

In ectropion, the cervix has a large area of red appearance around the external os and the squamocolumnar junction far away from the os. Nabothian cysts appear as bulging blue-white or yellow-white nodules, having a smooth delicate lining with branching blood vessels. In some

women, nabothian cysts can become large and distort the shape of the cervix. A cervical polyp appears as a smooth mass protruding from the cervical canal beyond the external os, which may appear dark red or pink-white. Sometimes a necrotic polyp resembles a cervical cancer. Healed lacerations appear as tears on the lips of the cervix, with the external os appearing irregular. Leukoplakia appears as a smooth-surfaced, white area on the cervix that cannot be removed or scraped off. Cervical condylomata appear as raised, grey-white areas within or outside the transformation zone in the squamous epithelium and may be accompanied by similar lesions in the vagina and vulva.

Look for small blisters containing fluid or multiple, small ulcers on the cervix. Extensive erosive red areas may be present on the cervix, extending to the vagina in instances of severe cervical infection and inflammation. Observe whether there is any bleeding from the cervix, especially on touch, or ulceroproliferative growth. A very early invasive cancer may present as a rough, reddish, granular area that may bleed on touch. More advanced invasive cancers may present as a large exophytic growth with an ulceroproliferative, bulging mass with polypoid or papillary excrescences, arising from the cervix or as a predominantly ulcerating growth replacing most of the cervix. In both of these types, bleeding on touch and necrosis are predominant clinical features. Foul-smelling discharge is also common due to superadded infection. Occasionally, invasive cancer can present as an infiltrating lesion with a grossly enlarged irregular cervix.

Now, gently, but firmly, apply 5% acetic acid using a cotton swab soaked in acetic acid. The secretions should be gently wiped

off. The swabs after use should be disposed of in the waste bucket. The curdy-white discharge associated with candidiasis is particularly sticky, and if particular care is not taken to remove it properly, it may mimic an acetowhite lesion, thus leading to a false-positive result. After removing the swab, carefully look at the cervix to see whether any white lesions appear, particularly in the transformation zone close to the squamocolumnar junction, or dense, non-removable acetowhite areas in the columnar epithelium. The results one minute after application of acetic acid should be reported. Note how rapidly the acetowhite lesion appears and then disappears.

Carefully observe:

- The intensity of the white colour of the acetowhite lesion: if it is shiny-white, cloudy-white, pale-white or dull-white.
- The borders and demarcations of the white lesion: distinctly clear and sharp or indistinct diffuse margins; raised or flat margins; regular or irregular margins.
- Whether the lesions are uniformly white in colour, or the colour intensity varies across the lesion, or if there are areas of erosion within the lesion.
- Location of the lesion: is it in, near or far away from the transformation zone? Is it abutting (touching) the squamocolumnar junction? Does it extend into the endocervical canal? Does it occupy the entire, or part of, the transformation zone? Does it involve the entire cervix (which usually indicates early preclinical invasive cancer)?
- Size (extent or dimensions) and number of the lesions.

If in doubt, it is safe to repeat the test a few times, taking care not to induce bleeding. Women with suspected invasive cancers should be referred for further investigations and treatment.

Conclusion of the examination

Contaminated swabs, gauze and other waste material should be disposed of in the plastic bag in the plastic bucket.

Withdraw the speculum gently, and inspect the vaginal walls for condyloma and acetowhite lesions. Before removing the soiled gloves, immerse the hands briefly in a container filled with 0.5% chlorine solution. Decontaminate the used gloves by soaking in the 0.5% chlorine in a plastic bucket for 10 minutes. Preparation of 0.5% chlorine solution is described in Appendix 4.

The speculum and other instruments used for VIA should be immersed in 0.5% chlorine solution for 10 minutes' decontamination, before cleaning with detergent and water. The cleaned instruments may be reused after high-level disinfection by immersing them in boiling water for 20 minutes or by sterilizing the instruments using an autoclave.

Documentation of findings and advising the woman

Carefully document the outcome of testing in the reporting form (Appendix 3). Explain the outcome of the test to the woman, as well as any further course of follow-up actions. If the test is negative, the woman is reassured and she may be advised to repeat testing after five years. If the test is positive, she should be referred for further investigations such as colposcopy and biopsy as well as treatment for any confirmed lesions. If invasive cancer is

suspected, she should be referred to a cancer diagnosis and treatment facility.

Reporting the outcome of VIA

VIA negative (-)

VIA screening is reported as negative in the case of any of the following observations:

- No acetowhite lesions are observed on the cervix (Figure 2.1).
- Polyps protrude from the cervix with bluish-white acetowhite areas (Figure 2.2).
- Nabothian cysts appear as button-like areas, as whitish acne or pimples (Figure 2.3).
- Dot-like areas are present in the endocervix, which are due to grape-like columnar epithelium staining with acetic acid (Figure 2.4).
- There are shiny, pinkish-white, cloudy-white, bluish-white, faint patchy or doubtful lesions with ill-defined, indefinite margins, blending with the rest of the cervix (Figures 2.5-2.7).
- Angular, irregular, digitating acetowhite lesions, resembling geographical regions, distant (detached) from the squamocolumnar junction (satellite lesions) (Figure 2.8).
- Faint line-like or ill-defined acetowhitening is seen at the squamocolumnar junction (Figures 2.8-2.10).
- Streak-like acetowhitening is visible in the columnar epithelium (Figure 2.8).
- There are ill-defined, patchy, pale, discontinuous, scattered acetowhite areas (Figures 2.10-2.11).

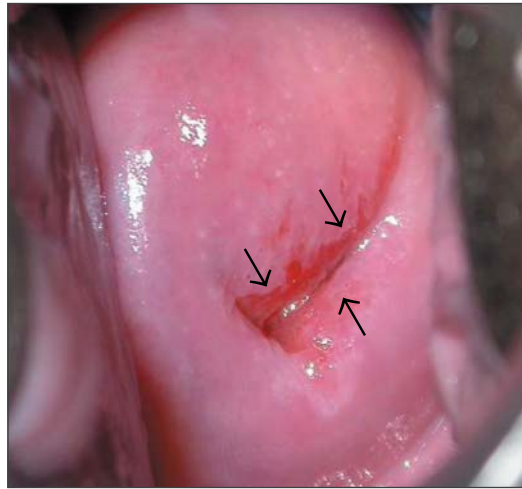


FIGURE 2.1:
VIA negative: No acetowhite area seen. Note the advancing edges of squamous metaplasia in the anterior and posterior lips (arrows).

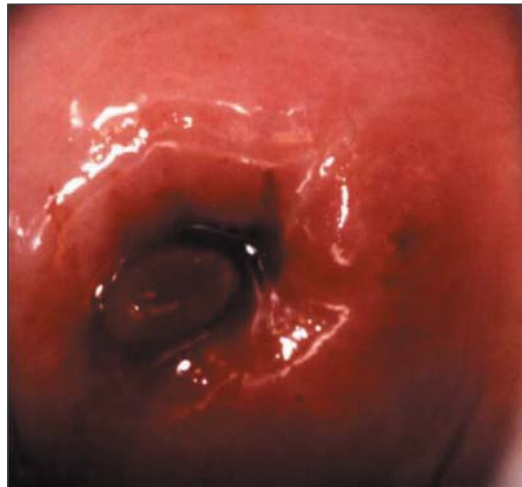


FIGURE 2.2:
VIA negative. There are no acetowhite areas on the polyp and the cervix after the application of acetic acid.



FIGURE 2.3:
VIA negative. The nabothian cysts appear as pimple- or button-like areas after the application of acetic acid.



FIGURE 2.5:
VIA negative: There are ill-defined pinkish-white and cloudy-white areas with indefinite margins blending with the rest of the epithelium. The squamocolumnar junction is fully visible.



FIGURE 2.4:
VIA negative: There is dot-like acetowhitening in the columnar epithelium in the anterior lip. The squamocolumnar junction is fully visible.



FIGURE 2.6:
VIA negative: There is an ill-defined pinkish-white hue with indefinite margins blending with the rest of the epithelium. The squamocolumnar junction is fully visible.



FIGURE 2.7:
VIA negative: There is an ill-defined pinkish-white hue, with indefinite margins, blending with the rest of the epithelium. The squamocolumnar junction is fully visible.

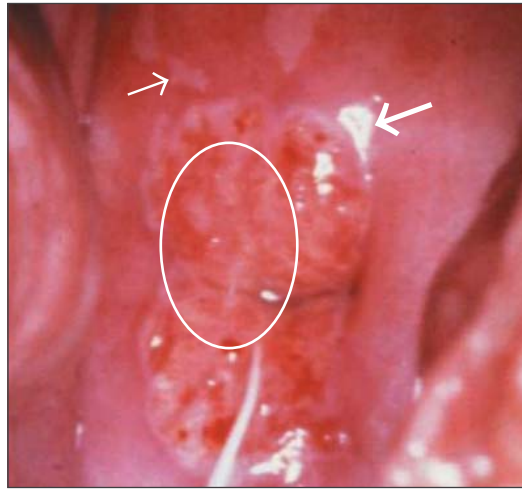


FIGURE 2.8:
VIA negative: There are pale white, satellite, geographical lesions with angular margins (narrow arrow) far away from the squamocolumnar junction (dense arrow). Note the streak-like acetowhitening in the columnar epithelium (within the oval area).



FIGURE 2.9:
VIA negative: There is dense, thick, mucus on the cervix before the application of acetic acid. After the application of acetic acid, the mucus is cleared and the squamocolumnar junction becomes prominent.



FIGURE 2.10:
VIA negative: The squamocolumnar junction is prominent after the application of acetic acid. Note the ectropion.

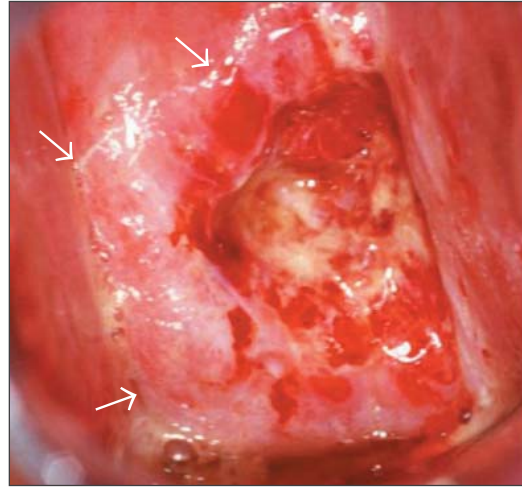


FIGURE 2.11:
VIA negative: The cervix is unhealthy, inflamed with ulceration, necrosis, bleeding and inflammatory exudate. There is ill-defined, diffuse, pinkish-white acetowhiteness with indefinite margins blending with the rest of epithelium (arrows).

VIA positive (+)

The VIA test outcome is reported as positive in any of the following situations:

- There are distinct, well-defined, dense (opaque, dull- or oyster-white) acetowhite areas with regular or irregular margins, close to or abutting the squamocolumnar junction in the transformation zone or close to the external os if the squamocolumnar junction is not visible (Figures 2.12-2.20).
- Strikingly dense acetowhite areas are seen in the columnar epithelium (Figures 2.21-2.22).
- The entire cervix becomes densely white after the application of acetic acid (Figure 2.23).
- Condyloma and leukoplakia occur close to the squamocolumnar junction, turning intensely white after application of acetic acid.



FIGURE 2.12:
VIA positive: There is a well-defined, opaque acetowhite area, with irregular digitating margins, in the anterior and posterior lips abutting the squamocolumnar junction and extending into the cervical canal.



FIGURE 2.13:
VIA positive: There is a well-defined, opaque acetowhite area, with bleeding on touch, in the anterior lip, abutting the squamocolumnar junction, which is fully visible.



FIGURE 2.15:
VIA positive: There is a well-defined, opaque acetowhite area, with regular margins, in the lower lip, abutting the squamocolumnar junction, which is fully visible.



FIGURE 2.14:
VIA positive: There is a well-defined, opaque acetowhite area, with regular margins, in the anterior lip, abutting the squamocolumnar junction, which is fully visible.



FIGURE 2.16:
VIA positive: There is a well-defined, opaque acetowhite area, with regular margins, in the anterior lip, abutting the squamocolumnar junction, which is fully visible. Note the satellite lesions in the lower lip.



FIGURE 2.17:

VIA positive: There is a well-defined, opaque acetowhite area, with regular margins, in the anterior lip, abutting the squamocolumnar junction, which is fully visible. Note the somewhat ill-defined white area in the lower lip. The lesion is extending into the cervical canal.



FIGURE 2.19:

VIA positive: There is a well-defined, dull, dense, opaque acetowhite area, with raised and rolled-out margins in the anterior lip abutting the squamocolumnar junction which is fully visible. The lesion is extending into the cervical canal.



FIGURE 2.18:

VIA positive: There is a well-defined, dull, dense, opaque acetowhite area in the anterior lip abutting the squamocolumnar junction which is fully visible.



FIGURE 2.20:

VIA positive: There is a well-defined, dull, dense, opaque acetowhite area in the posterior lip extending into the endocervical canal.

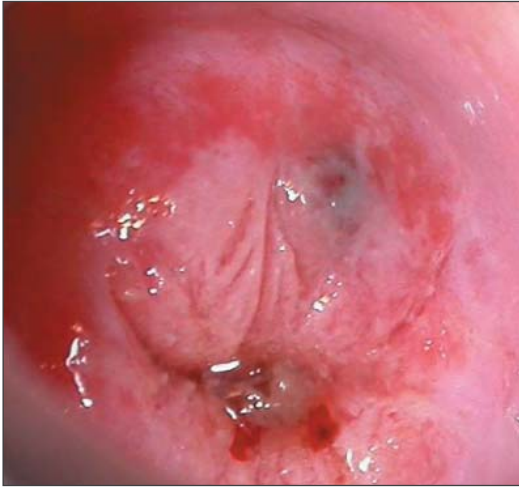


FIGURE 2.21:
VIA positive: There is an acetowhite area in the columnar epithelium in the anterior and posterior lips.

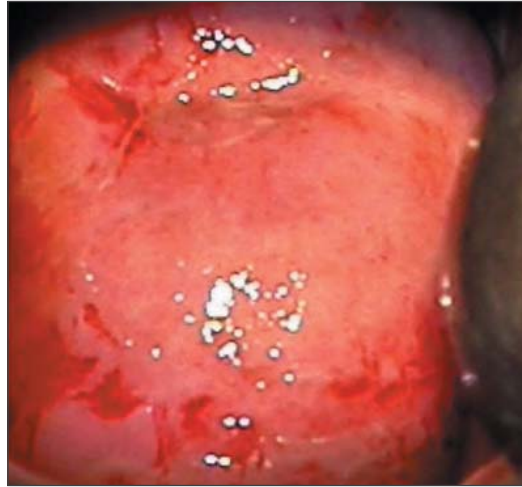


FIGURE 2.23:
VIA positive: There is a dense acetowhite area all over the cervix involving all the four quadrants and extending into the cervical canal.

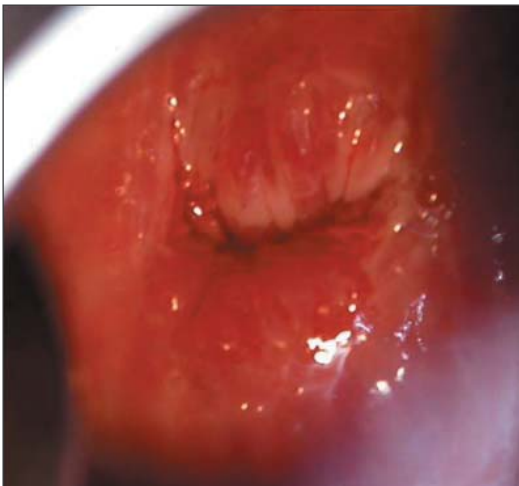


FIGURE 2.22:
VIA positive: There are dense acetowhite areas in the columnar epithelium in the anterior lip.

VIA positive, invasive cancer

The test outcome is scored as invasive cancer when:

- There is a clinically visible ulceroproliferative growth on the cervix that turns densely white after application of acetic acid and bleeds on touch (Figures 2.24-2.27).

Self-evaluation of the test providers

The test providers are encouraged to correlate the results of their VIA testing with those of colposcopy and histology. They are strongly advised to participate in the colposcopy sessions with doctors and review the findings. Such measures improve the skills of the test providers. A benchmark to assess one's own skill is to estimate what proportion of women examined are scored as aceto-positive and what proportion of aceto-positive



FIGURE 2.24:
VIA positive, invasive cancer: There is a dull, opaque, dense acetowhite area, with raised and rolled-out margins, irregular surface and bleeding on touch in the posterior lip. The lesion is extending into the cervical canal. The bleeding obliterates acetowhitening.



FIGURE 2.26:
VIA positive, invasive cancer: There is a dense acetowhite area with irregular surface contour.

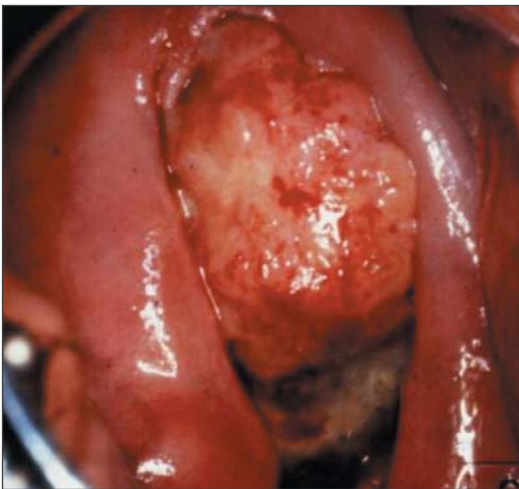


FIGURE 2.25:
VIA positive, invasive cancer: There is a proliferative growth with dense acetowhitening and bleeding.

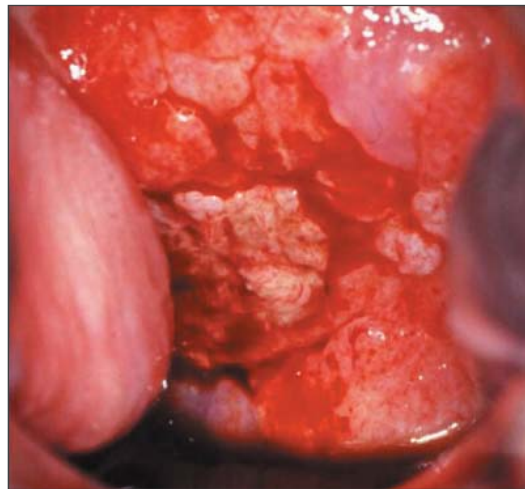


FIGURE 2.27:
VIA positive, invasive cancer: There is an ulceroproliferative growth with acetowhitening and bleeding.

women are ultimately diagnosed with CIN. A sufficiently skilled examiner will categorize 8-15% of women examined as aceto-positive, and 20-30% of the

acetowhite lesions identified on VIA by the test provider harbour CIN of any grade.

Chapter 3

Testing and reporting the results of visual inspection with Lugol's iodine (VILI)

Instruments and materials required:

- Examination table with knee crutches or leg rests or stirrups;
- Good light source (preferably a bright halogen lamp that can be easily directed at the cervix);
- Sterile speculum: Cusco's, Grave's or Collin's;
- Pair of gloves;
- Cotton swabs; cotton-tipped buds, gauze;
- Ring forceps, pickup forceps;
- 5% Lugol's iodine solution;
- An aluminium, steel or plastic container with 0.5% chlorine solution in which to immerse the gloves;
- Plastic bucket or container with 0.5% chlorine solution for decontamination of instruments;
- A plastic bucket with a plastic bag to dispose of contaminated swabs and other waste items.

Preparation of Lugol's iodine

Dissolve 10 g of potassium iodide in 100 ml distilled water. Add 5 g of iodine after the potassium iodide is fully dissolved. Stir well until all the iodine flakes have fully dissolved. The solution should be stored in a sealed container to prevent evaporation of iodine and loss of staining activity.

Test provider skills

The test provider must have a good knowledge on the anatomy, physiology and pathology of the cervix in relation to its visual examination. He/she should know the clinical features of benign conditions, inflammation, precancerous lesions and invasive cancer of the cervix.

Procedure

Women coming for testing should have the screening procedure explained to them in detail. Written informed consent should be obtained before screening. An example of a written informed consent form is given in Appendix 2. Relevant obstetric and gynaecological history should be obtained and recorded with the help of a form for this purpose (Appendix 3). The woman should be reassured that the procedure is painless, and every effort should be made to ensure that she is fully relaxed and remains at ease during testing.

The woman is invited to lie down in a modified lithotomy position on a couch with leg rests or knee crutches. After proper positioning of the woman, observe if there is any vaginal discharge. Observe the external genitalia, and perineal region for any signs of excoriations, oedema, vesicles, papules, sores,

ulceration and warts. Look for any swelling in the inguinal/femoral region. Then proceed to wipe the vaginal introitus and the surrounding perineum with a cotton swab or gauze soaked with an antiseptic solution.

Afterwards, gently introduce a sterile vaginal speculum, which has been immersed in warm water, and open the blades of the speculum to view the cervix. Adjust the light source so that there is adequate light in the vagina and on the cervix. As the speculum is gently opened and the lips are fixed, the cervix comes into view. Observe the size and shape of the cervix.

Identify the external os, columnar epithelium (red in colour), squamous epithelium (pink) and the squamocolumnar junction. Proceed to identify the transformation zone, the upper limit of which is formed by the squamocolumnar junction. Look for ectropion, cervical polyp, nabothian cysts, healed laceration of the cervical lips, leukoplakia, condylomata and signs of cervicitis. You may note that in postmenopausal women, the cervix appears pale and brittle, due to thinning and atrophy of the squamous epithelium. Assess the characteristics of discharge in terms of quantity, colour, odour and thickness. Thread-like, thin mucinous discharge from the external os indicates ovulation. Blood flow through the external os is observed in women during menstruation, and they may be subjected to VILI after 10-15 days.

In ectropion, the cervix has a large area of gross red appearance around the external os and the squamocolumnar junction far away from the os. Nabothian cysts appear as bulging blue-white or yellow-white nodules, having a smooth delicate lining with branching blood

vessels. In some women, nabothian cysts can become large and distort the shape of the cervix; however, they seldom ulcerate and become necrotic. A cervical polyp appears as a smooth mass protruding from the cervical canal beyond the external os, which may appear dark red or pink-white. Sometimes a necrotic polyp resembles a cervical cancer. Healed lacerations appear as tears on the lips of the cervix, with the external os appearing irregular. Leukoplakia appears as a smooth-surfaced, white area on the cervix that cannot be removed or scraped off. Cervical condylomata appear as raised, grey-white areas within, or outside, the transformation zone in the squamous epithelium and may be accompanied by similar lesions in the vagina and vulva.

Look for the presence of blisters with fluid or small ulcers on the cervix. Extensive erosive red areas may be present in the cervix, extending to the vagina in instances of severe cervical infection and inflammation. Observe whether there is any bleeding from the cervix, especially on touch, or ulceroproliferative growth. A very early invasive cancer may present as a rough, reddish, granular area, that may bleed on touch. More advanced invasive cancers may present as a large exophytic growth with an ulceroproliferative, bulging mass with polypoid or papillary excrescences, arising from the cervix or as a predominantly ulcerating growth replacing most of the cervix. In both of these types, bleeding on touch and necrosis are predominant clinical features. Foul-smelling discharge is also common due to superadded infection. Occasionally, invasive cancer can present as an infiltrating lesion with a grossly enlarged irregular cervix.

After carefully recording the visual findings, liberally and gently apply Lugol's iodine with a cotton swab on the cervix. Take care not to stain either the woman's or your own clothes with iodine! After removing the swab, carefully look at the cervix for any iodine non-uptake (non-staining) areas in the form of pale or yellowish-white areas, particularly in the transformation zone, close to the squamocolumnar junction. Once the examination is completed, the excess iodine in the vaginal fornices should be mopped up with dry cotton.

Conclusion of the examination

Contaminated swabs, gauze and other waste material should be disposed of in the plastic bag in the plastic bucket.

Withdraw the speculum gently, and inspect the vaginal walls for condyloma and iodo-negative lesions. Before removing the soiled gloves, immerse the hands briefly in a container filled with 0.5% chlorine solution. Decontaminate the used gloves by soaking in the 0.5% chlorine in a plastic bucket for 10 minutes. Preparation of 0.5% chlorine solution is described in Appendix 4.

The speculum and other instruments used during VILI should be immersed in 0.5% chlorine solution for 10 minutes' decontamination, before cleaning with water. The cleaned instruments may be reused after high-level disinfection by immersing them in boiling water for 20 minutes or by sterilizing the instruments using an autoclave.

Documentation of findings and advising the woman

Carefully document the outcome of testing in the reporting form (Appendix 3). Explain the outcome of the test to the

woman, as well as any further course of follow-up actions. If the test is negative, the woman is reassured and she may be advised to repeat testing after five years. If the test is positive, she should be referred for further investigations such as colposcopy and biopsy as well as treatment for any confirmed lesions. If invasive cancer is suspected, she should be referred to a cancer diagnosis and treatment facility.

Reporting the outcome of VILI

The patterns associated with the outcomes after VILI are shown in Figures 3.1-3.21.

VILI negative (-):

VILI screening is reported as negative in the case of any of the following observations after iodine application:

- A normal cervix; the squamous epithelium turns mahogany brown or black and the columnar epithelium does not change colour (Figure 3.2).
- Patchy, indistinct, ill-defined, colourless or partially brown areas are seen (Figures 3.3-3.6).
- Pale areas of no or partial iodine uptake are present on polyps (Figure 3.7).
- A leopard-skin appearance (Figure 3.8) is associated with *T. vaginalis* infection.
- Pepper-like non-iodine uptake areas are seen in the squamous epithelium, far away from the squamocolumnar junction (Figure 3.9)
- Satellite, thin, yellow, non-iodine uptake areas with angular, or digitating margins, resembling geographical areas, are seen far away from the squamocolumnar junction (Figure 3.10).

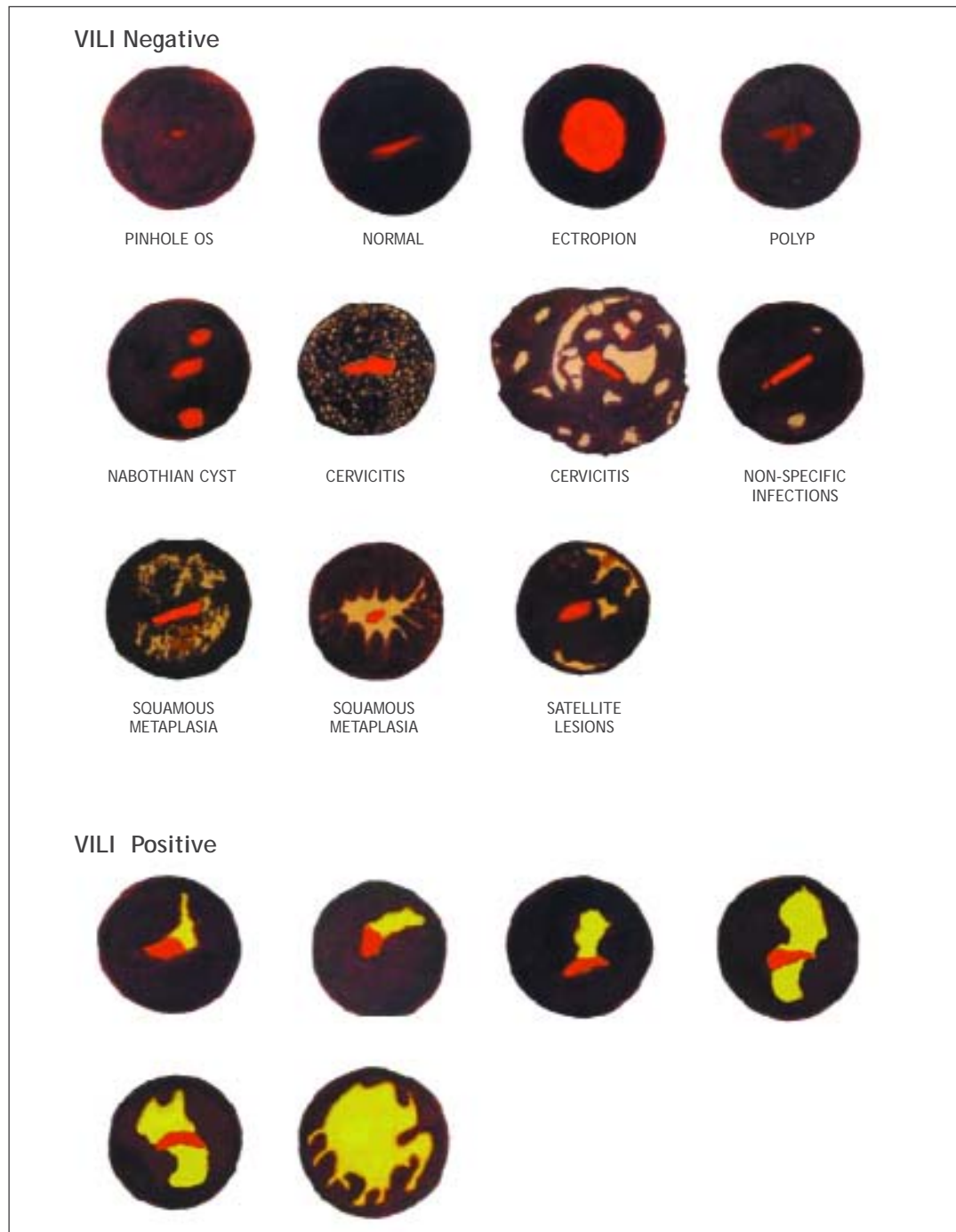


FIGURE 3.1:
Visual inspection with Lugol's iodine (VILI).

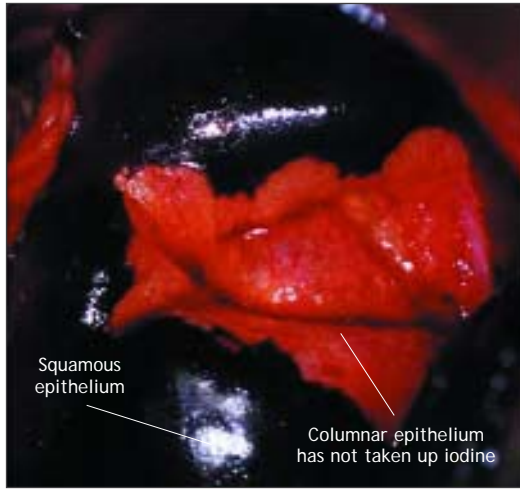


FIGURE 3.2:
VILI negative: The squamous epithelium is black and the columnar epithelium does not change colour after the application of iodine.

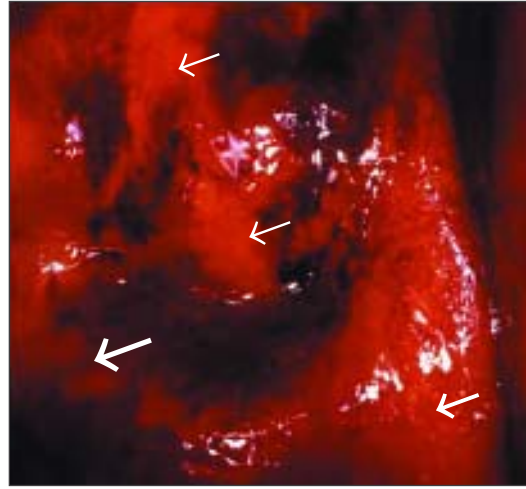


FIGURE 3.4:
VILI negative: There are patchy, ill-defined areas of iodine non-uptake (narrow arrows) and partial iodine uptake (dense arrows).

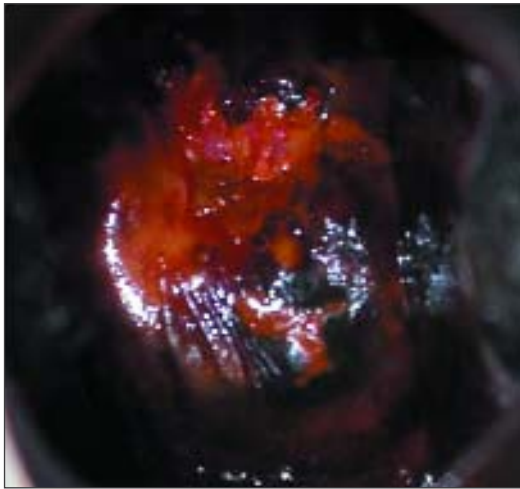


FIGURE 3.3:
VILI negative: There are patchy, ill-defined, scattered non-iodine uptake areas in the cervix; these are not restricted to the transformation zone. This appearance is due to inflammation of the cervix.

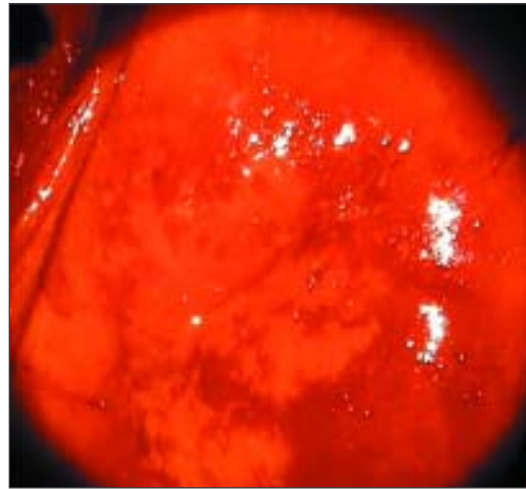


FIGURE 3.5:
VILI negative: Squamous epithelium remains brown. There are patchy areas of no or partial uptake of iodine in the transformation zone corresponding to areas of immature squamous metaplasia and inflammation.

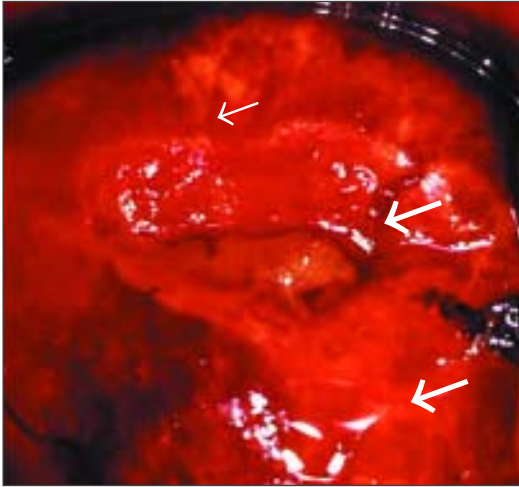


FIGURE 3.6:
VILI negative: There are patchy, ill-defined areas of iodine non-uptake (narrow arrow) and partial iodine uptake (dense arrows).



FIGURE 3.8:
VILI negative: There are patchy areas of iodine non-uptake, scattered all over the cervix, not restricted to the transformation zone. This is characteristic of chronic cervicitis.

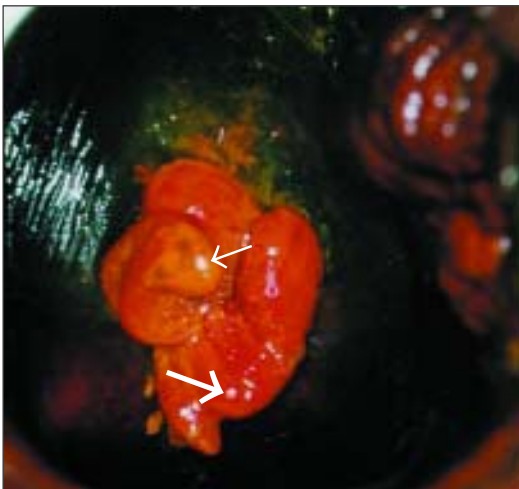


FIGURE 3.7:
VILI negative: There are areas of iodine non-uptake (narrow arrow) and partial uptake (dense arrow) in the polyp. Squamous epithelium is black.



FIGURE 3.9:
VILI negative: There are pepper-like areas of iodine non-uptake in the squamous epithelium, due to cervical ulceration following inflammation.

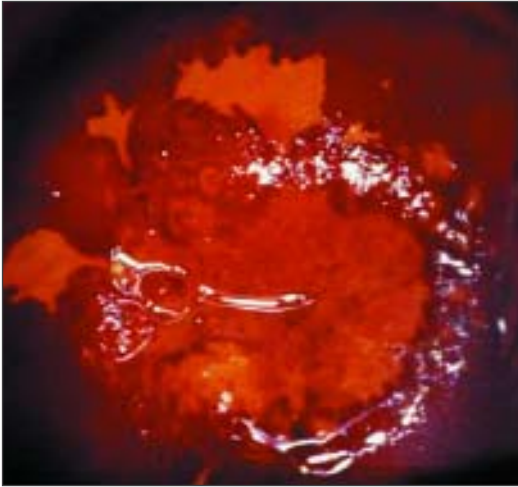


FIGURE 3.10:
VILI negative: Iodine-negative, irregular yellow areas are detached from the squamocolumnar junction and constitute 'satellite' lesions.



FIGURE 3.12:
VILI positive: There is a mustard-yellow lesion with no iodine uptake in the anterior lip touching the squamocolumnar junction.



FIGURE 3.11:
VILI positive: There is a saffron-yellow area of iodine non-uptake in the anterior lip abutting the squamocolumnar junction.

VILI positive (+):

The outcome is scored as positive if dense, thick, bright, mustard-yellow or saffron-yellow iodine non-uptake areas are seen in the transformation zone, close to or abutting the squamocolumnar junction or close to the os if the squamocolumnar junction is not seen (Figures 3.11-3.15) or when the entire cervix turns densely yellow (Figure 3.16).

VILI positive, invasive cancer:

Invasive cancer is reported when a frank, nodular, irregular, ulceroproliferative growth is visible on the cervix which turns densely yellow on application of iodine (Figures 3.17-3.19).

Self-evaluation of the test providers

The test providers are encouraged to correlate the results of their VILI testing with histological findings (if done). A sufficiently skilled examiner will categorize 10-15% of women as VILI +, and 20-30% of the women VILI+ harbour CIN of any grade.

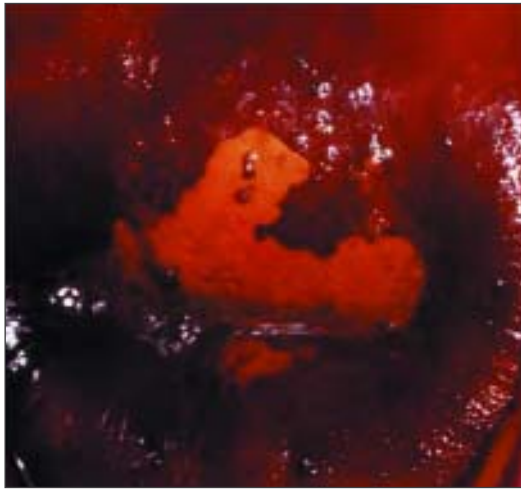


FIGURE 3.13:
VILI positive: There is a mustard-yellow area of iodine non-uptake in the anterior lip abutting the squamocolumnar junction.

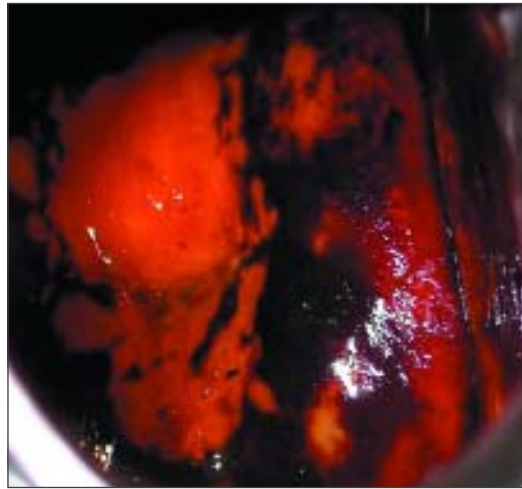


FIGURE 3.15:
VILI positive: There are large, thick, mustard-yellow areas of iodine non-uptake in the upper and lower lip of the cervix extending into the cervical canal.

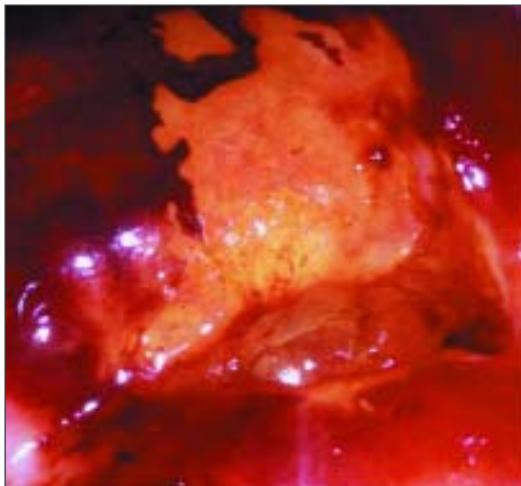


FIGURE 3.14:
VILI positive: There is a dense, mustard-yellow area of iodine non-uptake abutting the squamocolumnar junction in the anterior lip of the cervix with irregular, angular margins.

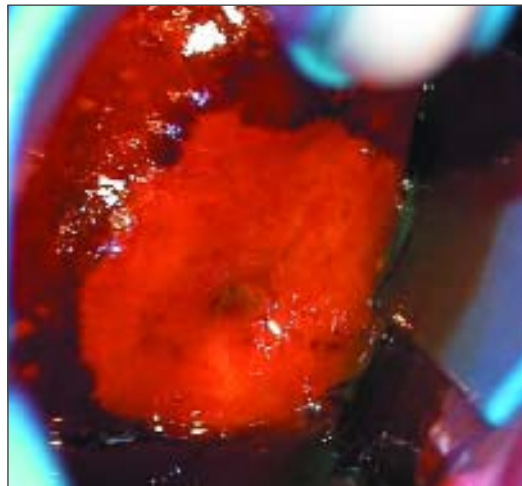


FIGURE 3.16:
VILI positive: There is a large, dense, saffron-yellow area of iodine non-uptake with irregular surface, involving all the four quadrants and extending into the cervical canal.

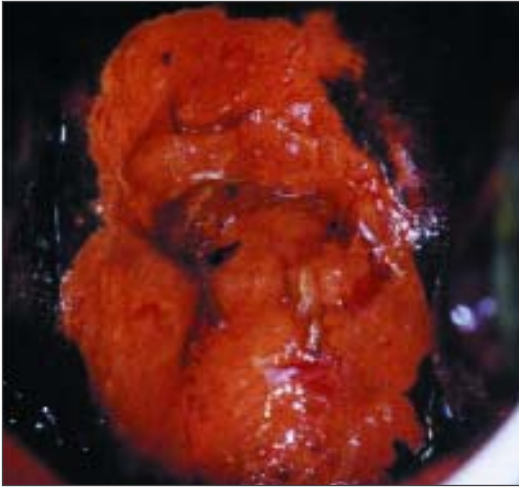


FIGURE 3.17:
VILI positive, invasive cancer: There is a dense, thick, extensive mustard-yellow area, with irregular and nodular surface, involving all the quadrants of the cervix. The external os is obliterated.

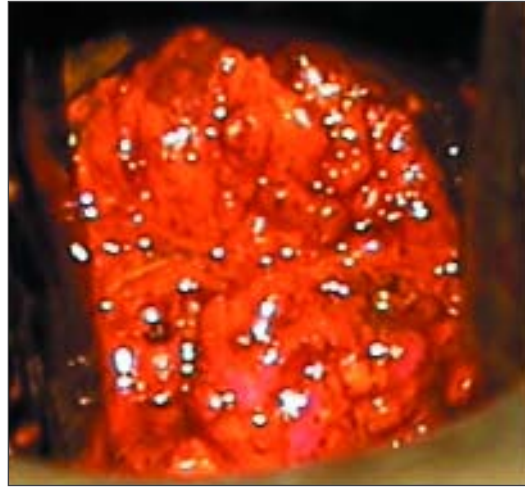


FIGURE 3.19:
VILI positive, invasive cancer: There is a large, thick mustard-yellow area in the cervix with irregular, nodular surface contour.

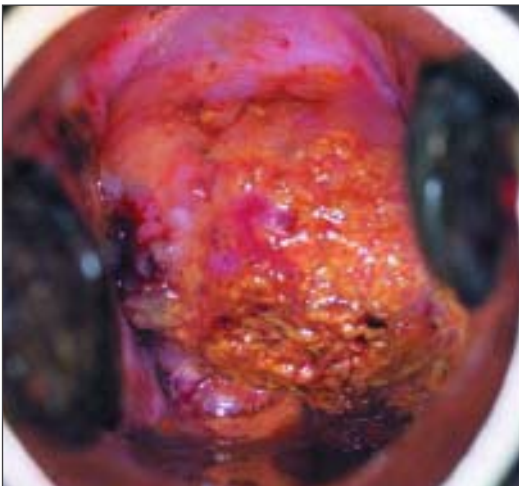


FIGURE 3.18:
VILI positive, invasive cancer: There is a large, thick, irregular, nodular mustard-yellow lesion suggestive of invasive cancer.

Appendix 1

FIGO staging of cervical carcinomas

Stage I

Stage I is carcinoma strictly confined to the cervix; extension to the uterine corpus should be disregarded. The diagnosis of both Stages IA1 and IA2 should be based on microscopic examination of removed tissue, preferably a cone, which must include the entire lesion.

Stage IA: Invasive cancer identified only microscopically. Invasion is limited to measured stromal invasion with a maximum depth of 5 mm and no wider than 7 mm.

Stage IA1: Measured invasion of the stroma no greater than 3 mm in depth and no wider than 7 mm diameter.

Stage IA2: Measured invasion of stroma greater than 3 mm but no greater than 5 mm in depth and no wider than 7 mm in diameter.

Stage IB: Clinical lesions confined to the cervix or preclinical lesions greater than Stage IA. All gross lesions even with superficial invasion are Stage IB cancers.

Stage IB1: Clinical lesions no greater than 4 cm in size.

Stage IB2: Clinical lesions greater than 4 cm in size.

Stage II

Stage II is carcinoma that extends beyond the cervix, but does not extend into the pelvic wall. The carcinoma involves the vagina, but not as far as the lower third.

Stage IIA: No obvious parametrial involvement. Involvement of up to the upper two-thirds of the vagina.

Stage IIB: Obvious parametrial involvement, but not into the pelvic sidewall.

Stage III

Stage III is carcinoma that has extended into the pelvic sidewall. On rectal examination, there is no cancer-free space between the tumour and the pelvic sidewall. The tumour involves the lower third of the vagina. All cases with hydronephrosis or a non-functioning kidney are Stage III cancers.

Stage IIIA: No extension into the pelvic sidewall but involvement of the lower third of the vagina.

Stage IIIB: Extension into the pelvic sidewall or hydronephrosis or non-functioning kidney.

Stage IV

Stage IV is carcinoma that has extended beyond the true pelvis or has clinically involved the mucosa of the bladder and/or rectum.

Stage IVA: Spread of the tumour into adjacent pelvic organs.

Stage IVB: Spread to distant organs.

Source: TNM Classification of malignant tumours. L. Sobin and Ch Wittekind (eds.), UICC International Union against Cancer, Geneva, Switzerland, pp155-157; 6th ed. 2002.

Appendix 2

INFORMED CONSENT

The doctor/health worker explained to me in detail about the vinegar (VIA)/iodine (VILI) test(s)* for the early detection and prevention of cancer in the neck of my womb (uterine cervix). I understand that the surface of my cervix will be visually inspected after application of 5% acetic acid/dilute iodine solution to detect or to exclude precancer/cancer. I understand that these procedures are generally harmless, but may occasionally cause some irritation or mild bleeding, which can be easily controlled.

I understand that, if the test is positive, other tests such as magnified inspection of the cervix with an instrument called a colposcope and examination of a sample of the tissue in my cervix (biopsy) may be recommended before treatment is provided. I have been informed that treatment by medicines or cryotherapy (destroying the diseased portion of the cervix by an ice-cold metal probe) or removing the diseased portion by minor surgery or major surgery and/or treatment with x-rays, may be required, in the event of any abnormality (infection or precancer or cancer or complications) being detected.

I hereby express my willingness to undergo the above tests and treatment, if advised.*
/ I am not willing to undergo the above procedures. *

Signature:

Date:

Name:

Address:

* Delete as appropriate

Appendix 3

Format for reporting results of VIA and VILI

Screening with VIA and VILI

1. Clinic/Serial/Unique number _____
2. Date of testing _____ [][]-[][]-[][]
(day (2 digits)-month (2 digits)-year (2 digits)):
3. Name: _____
4. Address: _____

5. Age (in years) _____ [][]
6. Education (1: Nil; 2: Primary; 3: Middle;
4: High school; 5: College; 9: Not known) _____ []
7. When did you have your last menstruation?
(1: Less than 12 months ago; 2: More than 12 months ago) _____ []
8. Marital status: (1: Married; 2: Widowed; 3: Separated;
8: Other; 9: Not known) _____ []
9. Age at marriage or first sexual intercourse: (99, if not known) _____ [][]
10. Total number of pregnancies/miscarriages: _____ [][]
11. Do you suffer from the following?
(use ✓ to indicate if the response is yes; otherwise, leave blank):
 - ☐ Excessive vaginal discharge
 - ☐ Itching in the external anogenitalia
 - ☐ Ulcers in the external anogenitalia
 - ☐ Lower abdominal pain
 - ☐ Pain during sexual intercourse

- ☐ Bleeding after intercourse
- ☐ Intermenstrual bleeding
- ☐ Low back ache

12. Visual examination findings

(use ✓ to indicate if the response is 'Yes', otherwise, leave blank):

- ☐ Squamocolumnar junction fully seen
- ☐ Cervical polyp
- ☐ Nabothian follicles
- ☐ Cervicitis
- ☐ Leukoplakia
- ☐ Condyloma
- ☐ Growth

13. Findings one minute after application of 5% acetic acid (VIA)

(1: Negative; 2: Positive; 3: Positive, invasive cancer) []

14. If VIA positive, does the acetowhite lesion extend into the endocervical canal?

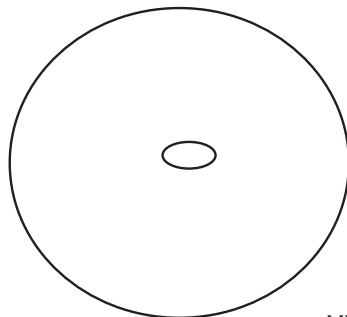
(1: Yes; 2: No) []

15. If VIA positive, how many quadrants are involved in the acetowhite lesion(s)?

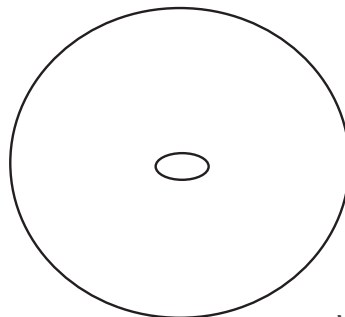
(1: Two or less; 2: Three; 3: Four quadrants) []

16. Diagram

(Draw the location of the squamocolumnar junction with a dotted line and the acetowhite/iodine non-uptake area(s) as a continuous line)



VIA



VILI

17. Findings after application of Lugol's iodine (VILI)

(1: Negative; 2: Positive; 3: Positive, invasive cancer) []

18. If invasive cancer, stage (1: IA; 2: IB; 3: IIA; 4: IIB;

5: IIIA; 6: IIIB; 7: IVA; 8: IVB; 9: Not known) []

19. Biopsy taken? (1: Yes; 2: No)
(If yes, indicate the biopsy site(s) in the diagram with 'x' mark) []
20. Action taken: (1: Advised follow-up after five years;
2: Advised medication for cervicitis and follow-up after six months;
3: Referred for colposcopy; 4: Referred for immediate treatment;
5: Referred for staging and treatment of invasive cancer;
6: Other, specify _____) []

Appendix 4

Cleaning and sterilization of instruments and materials used for early detection and treatment of cervical neoplasia:

Instrument/material	Processing	Suggested procedure
Vaginal speculum, vaginal retractors, biopsy forceps, toothed forceps, ring forceps, Cheatle's forceps.	Decontamination and cleaning followed by sterilization or HLD.	Decontamination by immersing in 0.5% chlorine for 10 minutes followed by cleaning with water and detergents; cleaned instruments may be immersed in boiling water for 20 minutes (high-level disinfection) or may be sterilized using autoclave before re-use.
Gloves.	Decontamination and cleaning followed by sterilization.	Decontamination by immersing in 0.5% chlorine for 10 minutes followed by cleaning with water and detergents; sterilized using an autoclave in wrapped packs.
Examination table, halogen lamp, torch lights, instrument trolley, trays.	Intermediate or low-level disinfection.	Wipe with 60-90% ethyl or isopropyl alcohol or with 0.5% chlorine solution.

HLD: High-level disinfection

Preparation of 0.5% chlorine solution:

The general formula for making a dilute chlorine solution from a commercial preparation of any given concentration is as follows: Total parts of water = [% concentrate/% dilute] - 1. For example, to make a 0.5% dilute solution of chlorine from 5% concentrated liquid household bleach = [5.0%/0.5%] - 1 = 10 - 1 = 9 parts of water; hence add one part of concentrated bleach to nine parts of water.

If one is using commercially available dry powder chlorine, use the following formula to calculate the amount (in grams) of dry powder required to make 0.5% chlorine solution:

Grams/litre = [% dilute/% concentrate] x 1000. For example, to make a 0.5% dilute

chlorine solution from a dry powder of 35% calcium hypochlorite = [0.5%/35%] x 1000 = 14.2 g. Hence add 14.2 grams of dry powder to 1 litre of water or 142 grams to 10 litres of water. The instruments should not be left in dilute bleach for more than 10 minutes and should be cleaned in boiled water immediately after decontamination to prevent discolouration and corrosion of metal.

Decontamination of the floor of the screening clinic:

The floor of the screening clinic should be decontaminated on a daily basis with chemical disinfectants including iodophores (e.g., 10% povidone iodine).

Appendix 5

Preparation of 5% acetic acid, Lugol's iodine solution, and Monsel's paste

5% dilute acetic acid

Ingredients

1. Glacial acetic acid
2. Distilled water

Quantity

- 5 ml
- 95 ml

Preparation

Carefully add 5 ml of glacial acetic acid into 95 ml of distilled water and mix thoroughly.

Storage: Unused acetic acid should be discarded at the end of the day.

Label: 5% dilute acetic acid

Note: It is important to remember to dilute the glacial acetic acid, since the undiluted strength causes a severe chemical burn if applied to the epithelium.

Lugol's iodine solution

Ingredients

1. Potassium iodide
2. Distilled water
3. Iodine crystals

Quantity

- 10 g
- 100 ml
- 5 g

Preparation

- A. Dissolve 10 g potassium iodide in 100 ml of distilled water.
- B. Slowly add 5 g iodine crystals, while shaking.
- C. Filter and store in a tightly stoppered brown bottle.

Storage: 1 month

Label: Lugol's iodine solution; Use by (date)

Monsel's paste

Ingredients

1. Ferric sulfate base	15 g
2. Ferrous sulfate powder	a few grains
3. Sterile water for mixing	10 ml
4. Glycerol starch (see preparation below)	12 g

Quantity

Preparation

Take care: The reaction is exothermic (emits heat).

- Add a few grains of ferrous sulfate powder to 10 ml of sterile water in a glass beaker. Shake.
- Dissolve the ferric sulfate base in the solution by stirring with a glass stick. The solution should become crystal clear.
- Weigh the glycerol starch in a glass mortar. Mix well.
- Slowly add ferric sulfate solution to glycerol starch, constantly mixing to get a homogeneous mixture.
- Place in a 25 ml brown glass bottle.
- For clinical use, most clinics prefer to allow enough evaporation to give the solution a sticky paste-like consistency that looks like mustard. This may take 2 to 3 weeks, depending on the environment. The top of the container can then be secured for storage. If necessary, sterile water can be added to the paste to thin it.

Note: This preparation contains 15% elementary iron.

Storage: 6 months

Label: Monsel's solution; Shake well; External use only; Use by (date)

Glycerol starch (an ingredient in Monsel's paste)

Ingredients

1. Starch	30 g
2. Sterile water for mixing	30 ml
3. Glycerine	390 g

Quantity

Preparation

- In a china crucible, dissolve the starch in the sterile water.
- Add the glycerine. Shake well.
- Heat the crucible and its contents over a bunsen burner. Mix constantly with a spatula until the mass takes on a thick, swelling consistency. Take care not to overheat so as not to let it turn yellow.

Storage: 1 year

Label: Glycerol starch; Store in a cool place; For external use only; Use by (date)

Note: Do not overheat, otherwise the mixture will turn yellow.

Suggestions for further reading

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