Taking better Pap smears

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An optimal clinical technique for Pap smear collection is necessary for accurate cervical cancer screening.

Even under the best of circumstances, screening is an inherently difficult process, fraught with potential errors of either nonrecognition of serious disease or mistaken recall of healthy individuals. In the field of cervical cytology these difficulties are readily amplified by any imperfections in the clinical technique of Pap smear collection. The NHMRC's *Guidelines for the management of women with screen detected abnormalities* presents an organised approach to screening for cervical cancer and evidence-based guidelines for evaluating and managing women with abnormalities detected by the screening program.[1] This article reviews the steps necessary to ensure optimal smear collection.

Cervical cancer screening

Over the past four decades, incidence and mortality rates for cervical cancer in Western countries have fallen by up to 70%. Limitations of the Pap smear notwithstanding, these improvements derive mainly from screening programs. Between 1990 and 2000 the age standardised incidence and mortality rates of cervical cancer each fell by 40% in NSW.[2] In the UK, the incidence of invasive cancer of the cervix fell 35% between 1985 and 1995.[3]

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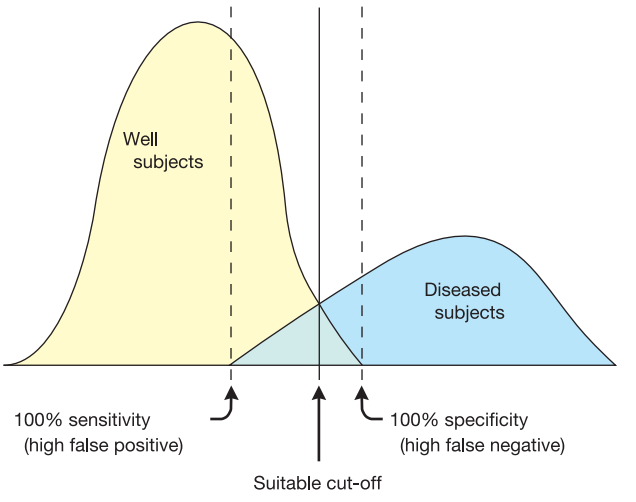
Worldwide, the interval cancer rate (i.e. cancers arising in adequately or even perfectly screened women occurring in the interval between screening examinations) is much greater than is generally believed. For example, 47 of 101 patients with cervical cancers seen during a three-year period at the University of Nebraska Medical School had had three or more smears in the previous five years. A population-based analysis of all cancers occurring in the State of Rhode Island over a five-year period substantiated concerns that interval cancers are most common in young women. Specifically, two-thirds of cancers in women over 40 years of age were attributable to lapsed screening, whereas two-thirds of cancers in women under 40 years occurred despite adequate screening.[4]

Problems with screening

For any given screening procedure, the

Screening tests

Screening tests are simple, quick procedures aimed at differentiating the 'apparently well' from the 'possibly diseased' in order to recall the latter for diagnostic assessment. Sometimes screening tests are clearly positive and are highly likely to indicate underlying disease. Other times screening tests are clearly negative and are very reassuring.



From the practical perspective, most of the problems with screening tests come from the 'grey area' formed by overlap in the distribution of 'apparently well' and 'probably diseased' subjects. In theory, one could abolish the grey area by moving the cut-off point to either the right or the left. However, 100% sensitivity results in the false positive recall of too many healthy women, and 100% specificity misses too many individuals with disease. Hence, the actual cut-off point is a compromise between ideal sensitivity and ideal specificity.

Common medicolegal allegations arising from cervical screening

Claims made against general practitioners (or other clinicians)

- Failure to offer cervical screening
- Failure to adequately investigate abnormal vaginal bleeding (especially postcoital bleeding)
- Failure to inform the patient of an abnormal result
- Failure to arrange adequate specialist referral for women with abnormal cytological results or a clinically suspicious cervix

Claims made against cytology laboratories

- Failure to detect, correctly grade or report the presence of abnormal cells on the Pap smear
- Failure to report a poor quality smear as unsatisfactory
- Failure to recommend an immediate repeat smear for specimens lacking an endocervical component

distributions of 'apparently healthy' and 'probably diseased' individuals overlap.[5] Unfortunately, sensitivity (ability to detect disease) is negatively correlated with specificity (ability to avoid falsely recalling healthy subjects), as shown in the box on page 59. Arithmetic principles thus dictate that no single test can ever deliver perfect performance. Rather, actual cut-off points must be chosen to give the best compromise between ideal sensitivity and ideal specificity.[6,7]

Traditionally, cytological cut-off points were set favouring specificity over sensitivity. The inevitable result was that a fraction of women with false negative smears would progress to cervical cancer before the next screening visit.

Litigation over missed cervical cancers following a false negative Pap smear has increased in Australia, following an exponential growth in the USA. Such litigation is irrational and does not take into account the inherent limitations of the screening process,[5] or the seemingly insoluble weaknesses of the conventional Pap smear – for example, nonrepresentative sampling, obscuring of abnormal cells by inflammatory cells or blood and misinterpretation or nonrecognition of certain morphological patterns of cervical intraepithelial neoplasia (CIN). Common medicolegal problems are listed in the box on this page.

Technological advances

Technological advances in Pap smear screening include the development of liquid based cytology (ThinPrep [Cytyc Corp] and AutocytePrep [Tripath]) and automated (computer assisted) screening. In the past five years, liquid based cytology specimens have shown excellent results with reports that at least 50% of laboratories in the USA are now replacing the conventional Pap smear with 'direct-to-vial' ThinPrep specimens. Liquid based cytology separates out excessive blood, mucus and inflammatory cells to produce an unobscured thin layer of epithelial cells for examination and is, therefore, particularly useful when a Pap smear has to be taken during menstrual bleeding and in women with vaginitis. In a large Australian study, Roberts and colleagues showed a 12% improvement in the detection rate of high grade epithelial abnormalities and a 95% reduction in unsatisfactory smear reporting rates when using the ThinPrep Pap test in addition to the conventional Pap smear.[8]

Computer assisted screening systems incorporating liquid based specimens are now being developed (ThinPrep Imaging System [Cytyc Corp] and Autopap Primary Screening System [Tripath]).

Automated computerised microscopic tracking (Comulyte Corp Pathfinder System), which automates the stage of the microscope to ensure no screening fields are missed, is another recent development.

At present, however, human interpretation of cytology specimens, whether conventional or liquid-based, is essential, even in computer assisted systems.

Screening guidelines

Sexually active women should be screened within two years of commencing sexual activity or at 18 years of age, whichever is the sooner. Virgins and lesbians are at low risk for cervical neoplasia, but should have a Pap smear if they so request. In women whose previous Pap smears have been consistently negative, screening can thereafter be performed every two years. Women with current or previous abnormalities need more frequent evaluation. Women can reasonably elect to discontinue cervical screening at the age of 70 years provided they have had at least two negative smears over the preceding five years. Women over 70 years who request Pap smears and those who do not fulfil the above requirements should continue in the screening program.

Post-hysterectomy screening

Hysterectomy protects most women from future cervical or vaginal intraepithelial neoplasia. Hence, Pap smears are no longer required in women in whom:

- hysterectomy has been performed for benign disease
- prior Pap smear history is known to be negative
- the cervix has been completely removed.

Women who have had a hysterectomy for benign disease but who are uncertain

of their prior screening history should have a baseline vault smear. If the smear is normal, then further screening is not needed.

Certain post-hysterectomy groups remain at substantial risk for vaginal malignancy and must remain in the screening program. These include:

- those with prior CIN
- those with vulvar intraepithelial neoplasia (VIN)
- immunosuppressed women
- those exposed to diethyl stilboestrol *in utero* (as they are at risk of the otherwise rare Müllerian tumour, clear cell carcinoma)
- those treated for prior pelvic malignancy.

The procedure

Timing of Pap smears

Ideally, Pap smears should be deferred until after the menses because of potential difficulties with interpretation. However, smears should certainly be taken in women who may not keep a follow up appointment and in women with very irregular menstrual patterns (as bleeding may reflect genital tract pathology rather than menses). When a Pap smear is taken during menstrual bleeding, a liquid based specimen in addition to the preparation of a conventional smear is very useful.

Likewise, in the presence of severe inflammation, it may well be better to defer cytology until after this problem has been treated, except where compliance is an issue (see the box on inflammatory smears on this page).

Women should avoid douching or using vaginal medications for 48 hours before their screening examination.

Atrophic cellular changes may make interpretation of the Pap smear difficult or even impossible. If possible, postmenopausal women with clinical evidence of cervical or vaginal atrophy should have a short course of oestrogen prior to their smear (oestriol [Ovestin Ovula Pessaries, 0.5 mg nocte; Ovestin Cream, 1 applicator – 0.5 mg – nocte]; oestradiol [Vagifem pessaries, 25 µg nocte]). Therapy should last for seven days, ceasing two days before the smear.

Instruments required

The following instruments are required to perform an adequate Pap smear:

- bivalve vaginal speculum (stainless steel or plastic disposable)
- adjustable light
- Cervex sampler or plastic Ayre spatula (the latter always in combination with an endocervical brush)
- slides (frosted tip)
- spray fixative or Coplin jar with 95% ethanol
- hard pencil.

Preparation

The slide should be ready and labelled before sampling. Marking of the frosted end should be done in pencil because ink and paper labels wash off during processing.

Filling out the request form is very important. Given the diagnostic difficulties of exfoliative cytology, it is essential that the pathologist be advised of the patient's age and date of last menstrual bleed, whether the patient is pregnant, postnatal, postmenopausal or using exogenous hormones, and whether there is an IUD in place. Each of these variables can confound cytological interpretation.

The pathologist should also be alerted to any high risk factors, such as prior CIN or treatment, an abnormal appearance of the cervix to the naked eye, or the presence of contact or postcoital bleeding. Smears from women with these risk factors will be double screened in high quality cytology laboratories.

Smear collection technique

Choice of speculum

Contrary to popular belief, the cervix does not form the apex of the vagina; rather, it is situated in the upper sixth of the anterior vaginal wall (Figure 1). Hence, the Graves and Pederson speculae are designed so that the lower blade is about 1 cm longer than the upper blade.

The Graves speculum gives the widest exposure of the upper vagina and cervix, and is the instrument of choice for colposcopic examination or cervical

Inflammatory smears

Inflammatory changes are not an index of pathology *per se*, but may prevent adequate smear interpretation or be a harbinger of associated disease.

Management is tied to the overall interpretation of the smear (negative, unsatisfactory, low grade epithelial abnormality, inconclusive, high grade epithelial abnormality).

- If the smear is reported as negative and satisfactory, the patient does not need to be recalled. Cytology is repeated at the scheduled time (in one to two years).
- If the degree of inflammation is sufficient to obscure cytological detail, the smear should be repeated in one to three months. Inflammation secondary to a specific organism (for example, *Candida* or *Trichomonas*) requires specific therapy. However, the use of sulfur-based creams for nonspecific inflammatory changes is illogical and a potential source of morbidity. Nonspecific inflammatory cells denote tissue repair rather than cervical infection.
- If the smear is reported as abnormal, referral for colposcopy is often the best response. A small but important fraction of inflammatory smears reflect tumour diathesis, arising from an occult invasive lesion.

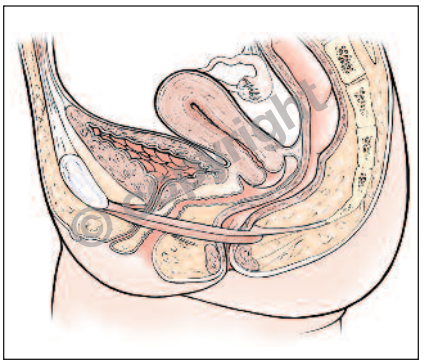


Figure 1. Normal vaginal anatomy. The vagina is not just a simple, straight tube. Rather, in the standing woman, the upper two-thirds of the vagina are oriented in a horizontal plane, and the lower third is oriented at about 45° to the vertical axis. The cervix is situated in the upper sixth of the anterior vaginal wall.

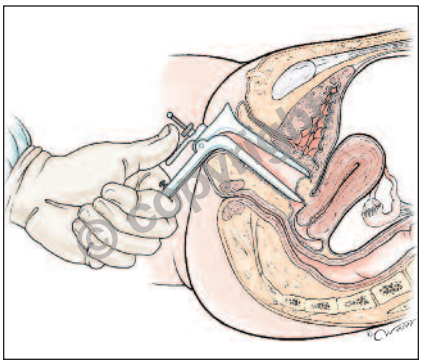


Figure 2a. When the physician is having trouble exposing the cervix, the most common reason (especially in nulliparous women) is failure to follow the almost horizontal axis of the upper vagina.

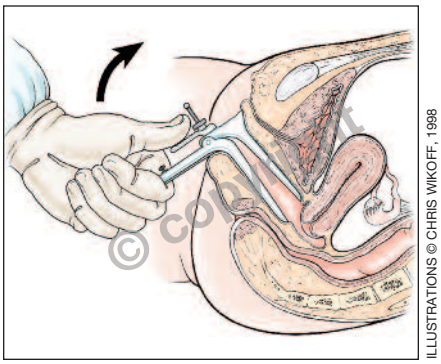


Figure 2b. The solution is to elevate the hand, thus pivoting the speculum across the perineal body and thereby depressing the speculum tips into the same axis as the cervix.

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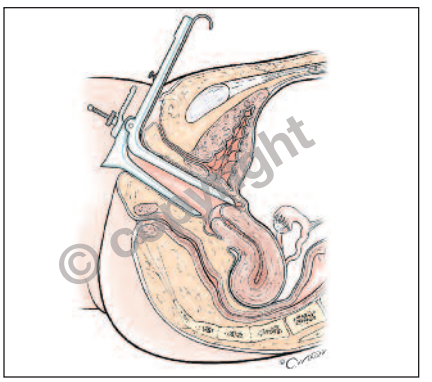

Figure 3. In patients with a retroverted uterus, the cervix points forward and upward (instead of backwards and down). Holding the speculum upside down so that the long blade of the speculum is along the anterior vaginal wall can help substantially with exposure.

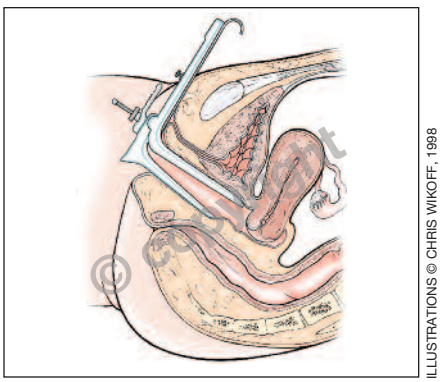

Figure 4. In patients with an anteverted uterus, attempting to insert the Graves speculum upside down makes cervical exposure difficult. The long blade pushes the cervix out of view, while the short blade fails to control bulging of the cul de sac and upper rectal wall.

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manipulation. However, for simple tasks like Pap smear collection the much narrower blades of the Pederson speculum make examination a lot more comfortable. The third speculum in common use is the Cusco, which has wide but easily inserted blades and no downward extension to catch on the examination couch. The Graves speculum is available in three sizes:

- small – used in children, women who have a very tight perineum, or aged patients with severe involution
- medium – used for most women
- large – used in morbidly obese women and grand multiparas.

Inserting the speculum

Pap smears should be collected before performing the bimanual examination. Lubricants should not be used, but passage of the speculum is facilitated by moistening it in lukewarm water.

Much of the discomfort of speculum insertion comes from pressure on the urethra and trigone. Therefore, the best technique of speculum insertion for cervical exposure is to align the transverse diameter of the instrument with the transverse diameter of the introitus. Insertion is then completed by angling the speculum downwards and backwards along the posterior vaginal wall with the tips pointing towards the rectal wall. Opening the bivalve speculum then allows the blades to slip easily into the anterior and posterior fornices, thus providing comfortable but efficient cervical exposure.

If difficulty is experienced in locating the cervix, the best initial strategy is to elevate your hand, thus directing the speculum blades further into the vaginal apex (Figures 2a and b). If this is not successful, the position and direction of the cervix can be established by gentle palpation with an index finger, and the speculum reinserted.

The Graves and Pederson speculae work most efficiently when examination is done on a specialised gynaecological couch fitted with stirrups. When using a standard plinth, insertion can be awkward because the vertical slide mechanism

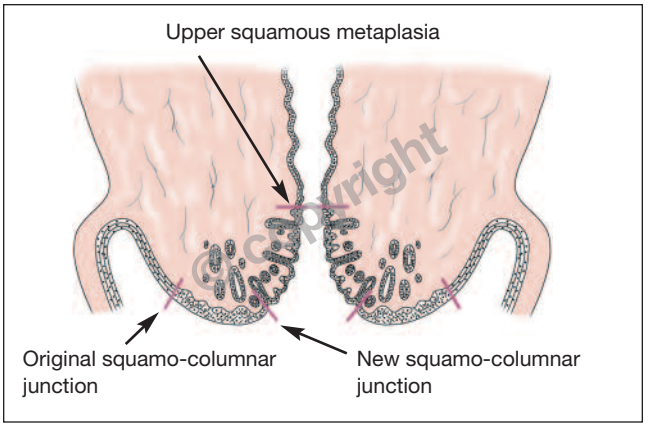

Figure 5a. In young women the transformation zone is well everted into the vaginal canal.

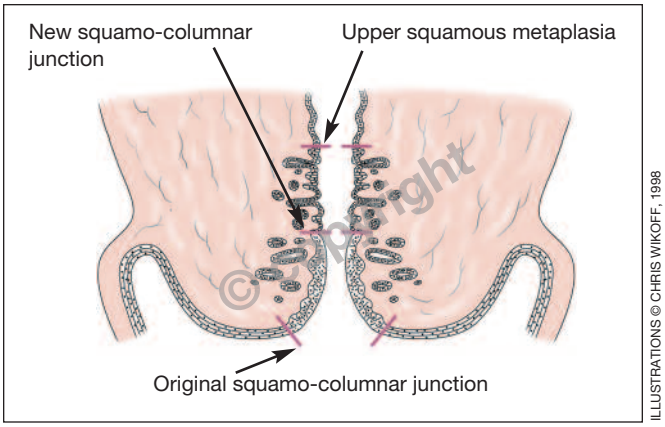

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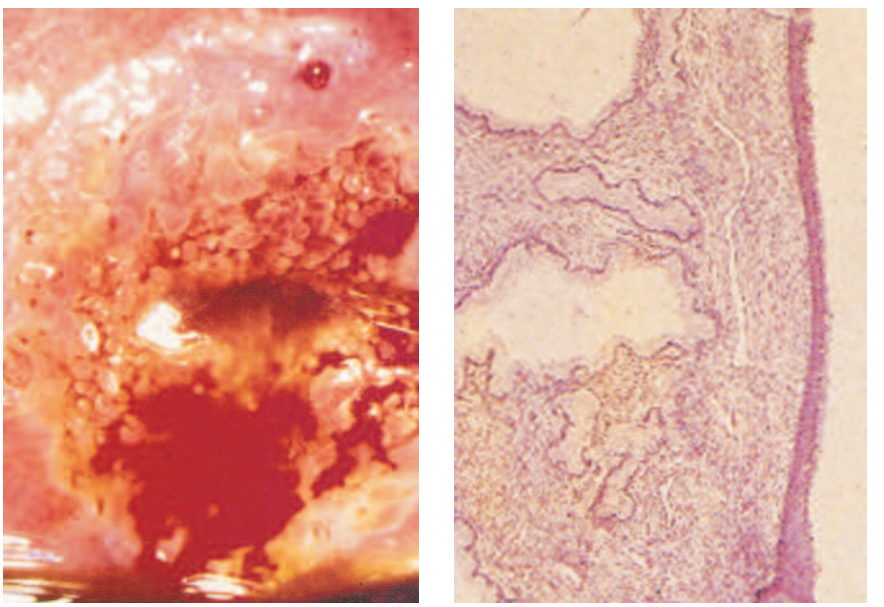
Figure 5b. As the cervical submucosa involutes with age, the transformation zone rolls back into the canal.

hits the mattress and prevents full depression of the hand holding the speculum. Such problems can be overcome by using a Cusco speculum, elevating the patient's hips with a plastic wedge (or telephone book), moving the patient into the lateral position, or holding the bivalve speculum upside down. The latter technique is certainly helpful in women with an acutely retroverted uterus, since the longer blade tends to deflect the cervix into a more visible position (Figure 3). However, when the uterus is anteverted, using the long blade of the speculum on the shorter vaginal wall can make for awkward mechanics (Figure 4).

If the problem is that the lateral vaginal walls are bulging inwards, consider using a larger size speculum (especially if the woman is obese) or applying a condom to the speculum (make a small scissor cut in the tip of the condom before reinserting).



Figures 6a and b. a (above left). Colpophotograph showing a circumferential, partially mature transformation zone that has been biopsied at '6 o'clock'. Squamous maturation of surface metaplastic epithelium is well advanced. b (above right). Biopsy photograph. Residual glandular elements are seen beneath the field of surface squamous metaplasia. There is a small zone of native (vaginal) squamous epithelium at bottom and a small zone of unchanged columnar epithelium at top.

Sampling the cervix

Most cancers and precancers arise in the transformation zone – that is, where the everted endocervical epithelium becomes replaced by metaplastic squamous epithelium (Figures 5a and b, 6a and b). Hence, this area must be selectively sampled.

Historically, Pap smears were collected using a wooden Ayre's spatula and a moistened cotton swab. However, numerous laboratory studies have shown that the porosity of wood and cotton mean that cells collected from the cervix are not transferred to the slide. While plastic Ayre's spatulas are better, there is good evidence that the Cervex brush (short for cervix examination brush) is the best tool in terms of both efficient sampling and reliable cell transfer (Figure 7). If a plastic Ayre's spatula is used, an endocervical brush sample

should always be taken as well.

In premenopausal women with an adequate external os, only the Cervex brush is needed. However, in postmenopausal women and in premenopausal women who have been treated for a cervical abnormality, an endocervical brush (for example, the Cytobrush) sample of the lower canal is also required. The Cervex brush should be rotated firmly at least once with the central longer bristles in the canal. The endocervical brush should be rotated for one-quarter of a turn only; more rotation than this drives the desired cells deeper into the bristles, and they may not be transferred to the glass slide. The Cervex brush sample should precede that of the endocervical brush.

Slight bleeding is to be expected, especially after taking an endocervical sample. This blood usually will not interfere with interpretation. Similarly, a small amount of mucus will not interfere with interpretation, but a large plug of mucus obscuring the external os should be removed gently with a swab before taking the smear.

Spreading the sample on the slide

One slide is sufficient, even when using the combined Cervex brush and endocervical brush technique. If just the Cervex brush is used, the cytological sample is transferred using a painting action, with just sufficient pressure to ensure cell transfer. The best technique is to paint the brush longitudinally down one side of the slide, rotate the brush through 180°, and then paint the other side (Figure 8). Partial overlap will occur, but causes no problems in laboratory interpretation.

If both the Cervex brush and the endocervical brush are used, you can choose between two techniques. Either roll the endocervical brush longitudinally down the slide after spreading the Cervex brush as previously described, or paint different ends of the slide with each instrument (Figures 9a and b).

Fixing the slide

The smear must be fixed quickly to prevent air drying, which can occur within 30 seconds (especially in summer). Air drying before fixation leads to degenerative changes with loss of the cellular features on which cytodiagnosis is based.

Most clinicians use an aerosol or pump action alcohol spray. The ideal technique is to give two sprays from a distance of 10 to 15 cm. Spraying closer can cause artefacts.

Reasons for screening failures

Recruitment failure

Public health programs promoting community awareness of the importance of cervical smears are important. Standard tactics have concentrated on educating women about Pap smears, and on warning of the dangers of lapsed screening. This approach is in stalemate. Of the women who develop invasive cervical carcinoma in Western society, most will have had other medical contact within this time period. Some may have resisted having a Pap smear because of discomfort or fear; others may not have been offered a smear. It is important that clinicians ask whether cervical screening is up to date.

Sampling collection failure

Many false negative smears arise because there are few abnormal cells present on the slide. This may be due to the clinician's failure to scrape the abnormal area of the cervix, or because a lesion failed to exfoliate despite being adequately sampled. Such sampling errors will be minimised by accurately targeting the transformation zone and a good clinical technique.

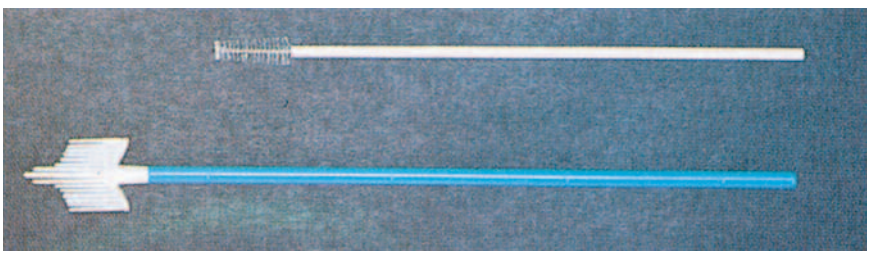


Figure 7. Cervex brush (bottom) and endocervical brush (top).

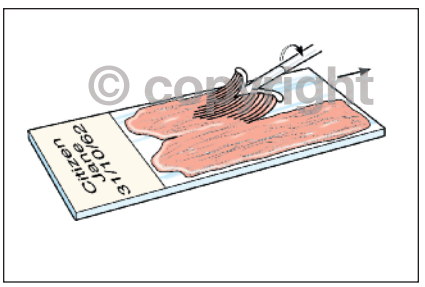
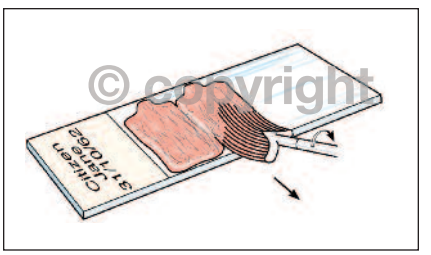
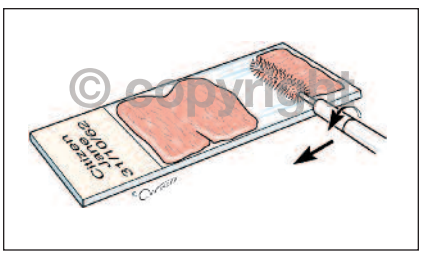


Figure 8. Slide preparation with Cervex brush alone.





Figures 9a and b. Single slide preparation using Cervex brush and endocervical brush. a (left). Paint one end of the slide with the Cervex brush. b (right). Paint the other end of the slide with the endocervical brush.

Transfer failure

Only about 20% of the cells collected are usually transferred to the slide when preparing a conventional smear. False negative cytology may, therefore, arise because abnormal cells present on the screening implement do not transfer to the slide or because the 20% of cells that did transfer are poorly representative of the collected cell sample. Liquid based cytology substantially reduces this source of error.

Laboratory screening failure

Abnormal cells present on the slide may be missed or misinterpreted during screening in the laboratory. This is more likely if the smear is of poor quality (Figure 10), if the number of abnormal cells is small or if the cells are distorted because of air drying. Problems can be minimised by circumferential sampling and rapid fixation.

The process of screening can be likened to finding a needle in a haystack. Even in good quality smears, abnormal cells are sometimes missed by well trained staff; even when located, abnormal cells may be misinterpreted as normal components of the smear.

The first safeguard against laboratory error is to select a laboratory with high standards and comprehensive quality assurance programs. The second is conscientious completion of the request form to alert the laboratory to the presence of abnormal cervical signs and symptoms in the patient; such smears will generally be double screened, and possibly checked by other measures. Thirdly, additional liquid based specimens can further reduce this source of error, although these tests generate additional costs for which currently there is no Medicare rebate.

Notification failure

Sometimes abnormal cells are correctly identified by the laboratory but do not come to the attention of the patient. The best safeguard against this source of error is for the doctor and patient to have agreed upon a plan as to how and when the results will be given. In the current medicolegal climate, it is wise that the doctor does not take sole responsibility for result notification. Thus, doctors should avoid telling the patient: 'If you don't hear anything, assume that your smear was negative'. Rather, the woman should share responsibility for recovery of the results, such as by agreeing to receive a mailed summary of the Pap smear report at her home. If the patient does not wish to receive notification by post, it should be documented in her file that she knows to telephone the surgery for the results.

The Pap test registers in each State or Territory have follow up protocols in place, with doctor and patient reminders that complement the follow up systems of doctors and pathology practices. Patients may 'opt off' these registers if they wish.

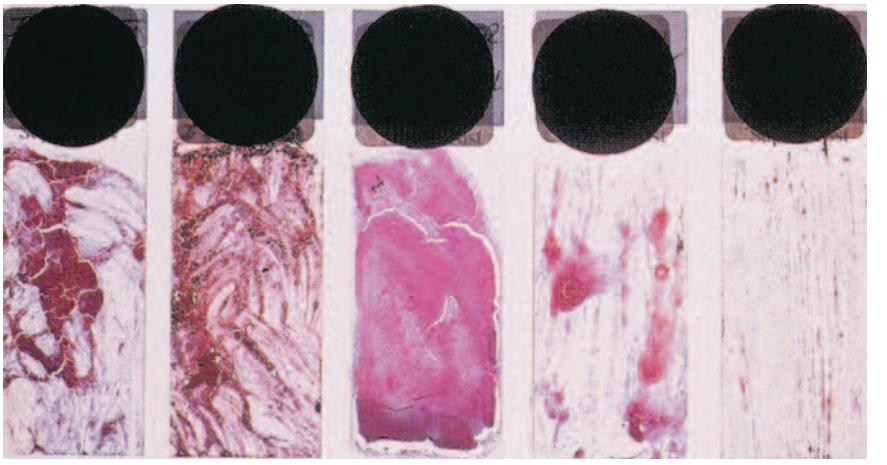


Figure 10. Four unsatisfactory smears – note the obscuring blood and inflammation – and an optimal slide (far right) for comparison.

When to do a repeat smear

Clinicians should wait at least six weeks before performing a repeat smear (for example, after a technically unsatisfactory smear), with a usual recommendation of three months. Before this time, the scraped surface may not have re-epithelialised and the chance of a false negative smear will be increased.

When to refer for colposcopy

If a Pap smear is reported CIN1, 'inconclusive' or 'high grade epithelial abnormality', the next step is referral for colposcopy rather than repeat cytology. Likewise, in patients with a macroscopically suspicious cervix, cytology cannot be relied on to rule out an occult cervical cancer. All such cases must be referred for colposcopy.

Conclusion

Accurate cervical cancer screening relies on the best possible smears being provided to the pathology laboratory for examination. Although screening is based on recommended practice, many litigation cases are arising over missed cervical cancers following cervical screening. The recently developed liquid based cytology techniques and the use of Cervex and endocervical brushes for sampling have increased the detection rate of high grade epithelial abnormalities and reduced the numbers of unsatisfactory smears but interpretation of cytology specimens still relies on humans, even in computer assisted systems. MT

A list of references is available on request to the editorial office.

Medicine Today 2004; 5(1): 59-65

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