

PAP SMEARS: TECHNIQUES, INTERPRETATION OF RESULTS AND MANAGEMENT OF ABNORMAL RESULTS

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ABSTRACT

Cytological examination of the cervix is a widely accepted method for detecting pre-invasive and early invasive lesions, before these lesions become symptomatic. For this test, epithelial cells are sampled from the transformation zone of the cervix with a special sampling device and examined with a microscope. Abnormal cells in the sample can be recognized microscopically by specially trained and experienced cytotechnologists. Early detection and treatment of cervical intraepithelial neoplasia (CIN) lesions by minimally invasive procedures can reduce morbidity. The correct sampling of a cervical smear with appropriate equipment contributes to a significant extent to the diagnostic value of the Pap test. The laboratories are encouraged to use the Bethesda system for reporting. There is a component related to the adequacy of the sample. This is the lab's assessment of how good the sample was by the time it arrived on their end. All slides are screened by a cytology screener. Smears which are thought to be abnormal are screened again by senior laboratory staff and are given a result code which depends on the degree of abnormality seen. Strict quality assurance procedures are adhered to; including rapid review by a senior member of staff of all smears originally classed as negative. At least 90% of CIN lesions can be removed under local anaesthesia. This can more or less guarantee that the individual woman will not die from cervical cancer.

OVERVIEW

Cytological examination of the cervix is a widely accepted method for detecting pre-invasive and early invasive lesions, before these lesions become symptomatic. For this test, epithelial cells are sampled from the transformation zone of the cervix with a special sampling device and examined with a microscope. Abnormal cells in the sample can be recognized microscopically by specially trained and experienced cytotechnologists. Early detection and treatment of cervical intraepithelial neoplasia (CIN) lesions by minimally invasive procedures can reduce morbidity. Thus development of cancer can be prevented.

METHODS OF SAMPLE COLLECTION

The quality of the cervical pap smear depends on a variety of factors; including sampling of specimen. The correct

sampling of a cervical pap smear with appropriate equipment contributes to a significant extent to the diagnostic value of the test. An inadequate smear is an important cause of false negative and false positive results.

The precursors of cervical cancer arise mainly in the transformation zone. This is the area between the squamous epithelium and the columnar epithelium. It is composed of columnar epithelium which has descended on to the ectocervix. Subsequently, this columnar epithelium metaplastes to a varying degree to squamous epithelium. This process exposes it to neoplastic transformation. Thus, it is important that cell material be sampled primarily from this zone. Sampling the transformation zone may be carried out using a cervical broom (Cervex-Brush®). The endocervical canal can be sampled using the endocervical brush (Cyto-brush®) (Fig 1).



Figure 1. Sampling devices: b. endocervical brush; c. cervical broom

TECHNIQUES OF SLIDE PREPARATION

Cervical broom (Cervex-Brush®)

Endocervical cells and exocervical cells are sampled simultaneously - the long bristles pick up endocervical cells while the short bristles collect exocervical cells (Fig 2).

- The long bristles are positioned endocervically.
- Rotate the brush five times over 360° with gentle pressure by rolling the handle clockwise between thumb and forefinger.
- Sweep the broom lengthwise along the slide, turn over and repeat for the other side.



Figure 2. Cervical broom : sampling and spreading the sample on the slide

Endocervical brush (Cyto-brush®)

- Insert the endocervical brush for two thirds into the endocervical canal, so that the lower bristles are still visible, and rotate gently 90 to 180°. (Fig 3)
- Roll (not wipe) the endocervical brush immediately over the outer third of the slide in the opposite direction from which it was collected by twirling the handle.
- Do the rolling in a single movement (not in a zigzag) and without pressure, in order to obtain a thin and even smear.

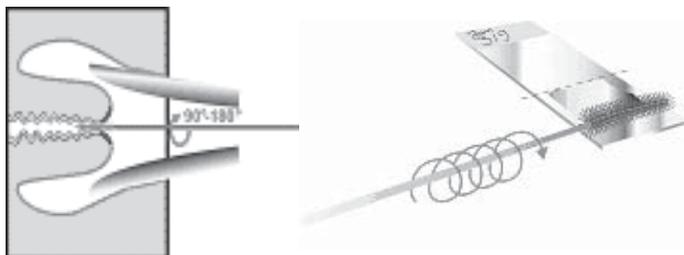


Figure 3. Sampling of cellular material using the cytobrush and spreading of sample on slide

Immediate fixation

The fixative of choice is 95% ethyl alcohol but other appropriate fixatives may be used. The smear should be sprayed with an aerosol fixative (Fig 4). It should be fix immediately by spraying at a right angle from a distance of 20 cm. If closer, the cells are blown away or frozen, if on a slant, the material is blown into aggregates. Avoid droplet formation: so do not use too much fixative. A very fast fixation, within a few seconds, is essential to prevent drying artefacts.



Figure 4. Fixation of the smear with spray

WHAT PROCESSES TAKE PLACE IN THE LABORATORY LOCALLY

The laboratories are encouraged to use the Bethesda system for reporting. There is a component related to the adequacy of the sample. This is the lab's assessment of how good the sample was by the time it arrived on their end.

All slides are screened by a cytology screener. Smears which are thought to be abnormal are screened again by senior laboratory staff and are given a result code which depends on the degree of abnormality seen. Strict quality assurance procedures are adhered to; including rapid review by a senior member of staff of all smears originally classed as negative.

The results are then sent to the smear taker.

INTERPRETATION OF RESULTS

There is a diversity of reporting schemes; ranging from the old pap system to CIN/dysplasia system to the Bethesda system (Table 1).

Table 1. Map of Pap smear classification schemes

Bethesda System					
Negative for malignancy (include reactive & infection)	ASCUS ASCH	LSIL	HSIL		Carcinoma

Dysplasia / SIN System					
Normal	Inflam / Atypia	Mild dysplasia / CIN 1	Moderate dysplasia / CIN 2	Severe dysplasia / CIN 3	Cancer

Old Pap System				
Class 1	Class 2	Class 3	Class 4	Class 5

- ASCUS – Atypical Squamous Cells of Undetermined Significance
- ASCH – Atypical Squamous Cells and cannot exclude high-grade lesion
- LSIL – Low-grade Squamous Intraepithelial Lesion
- HSIL – High-grade Squamous Intraepithelial Lesion

In the Bethesda system, there is a component related to the adequacy of the sample. It is to determine if the smear can be interpreted. If not, the reason for its rejection must be precisely indicated and with recommendations to improve the quality of the repeated smear. A cervical smear may be unsatisfactory for a variety of reasons. These include insufficient squamous epithelial cells, poor fixation, marked cytolysis or abundant neutrophils or blood obscuring more than 75% of the squamous cells present. Referral for colposcopy should be considered after three consecutive unsatisfactory smears.

Ninety percent of the results will turn out to be normal. This will be reported as negative for intraepithelial lesion or malignancy when there is no cellular evidence of neoplasia. Inflammation of the cervix is common, and some neutrophils are present in most cervical smears. When neutrophils are abundant, the squamous cell component may be obscured, resulting in satisfactory but limited smears, or unsatisfactory smears.

Inflammatory cells may be associated with a specific infectious agent eg. *Candida*, *Trichomonas*, bacterial vaginosis, *Actinomyces*. The organisms may be apparent in the smear. However, in many women, specific microbiological culture will be required to identify the microorganism responsible. The presence of inflammatory cells may or may not be associated with reactive epithelial cell changes. These are atrophic squamous cells, reaction to trauma, radiation or due to presence of intrauterine contraceptive device (IUD).

Epithelial cell abnormalities can be due to squamous cells or glandular cells. For squamous cells abnormality, these include atypical squamous cells of undetermined significance (ASC-US), atypical squamous cells cannot exclude high grade (ASC-H), low grade squamous intraepithelial lesion (LSIL), high grade squamous intraepithelial lesion (HSIL) and squamous cell carcinoma.

Among the glandular cells abnormalities, these include atypical glandular cells of undetermined significance (AGUS), atypical glandular cells and favor neoplastic, adenocarcinoma in situ (AIS) and adenocarcinoma.

MANAGEMENT OF ABNORMAL RESULTS

ASCUS encompasses a variety of squamous cellular changes which cannot be specifically classified. These changes exceed the features usually expected in benign reactive processes but are insufficient for a diagnosis of HPV or squamous intraepithelial lesion (SIL). An ASCUS report identifies a patient who is at risk for SIL. For women with a persisting abnormality, a significant number show SIL on colposcopic biopsy, which is usually low grade but may be high grade. In ASC-US, there is a 5% to 17% chance of having CIN 2,3 confirmed by biopsy. While CIN 2,3 is identified in 24% to 94% of those with ASC-H. However, the risk of invasive cervical cancer in a woman with ASC is low (approximately 0.1% to 0.2%). Therefore, women with ASC-H or 2 consecutive ASCUS should be referred for colposcopy.

Approximately 2% of the cervical smears will be reported as low-grade squamous intraepithelial lesions (LSIL). This category encompasses CIN 1 and/or a definite diagnosis of HPV. A proportion of smears reported as LSIL will have histologically proven high grade abnormalities. Immediate colposcopy following a single LSIL smear result allows early histological diagnosis and avoids the possible risk of the patient defaulting.

Women with a cytological diagnosis of high grade squamous intraepithelial lesion (HSIL) have approximately a 70% to 75% chance of having biopsy-confirmed CIN 2,3 and a 1% to 2% chance of having invasive cervical cancer. They should be referred directly for colposcopy.

The AGC category is associated with a substantially greater risk for cervical neoplasia than the ASC or LSIL categories. In follow-up studies of patients who have 'atypical glandular cells' reports, squamous lesions are the commonest abnormalities detected on biopsy. Adenocarcinoma-in-situ or adenocarcinoma is found in a minority of cases.

Women whose smears show squamous carcinoma, adenocarcinoma or any other malignant neoplasm should be referred immediately to an experienced colposcopist.

All abnormal pap smears require further evaluation, such as visual inspection of the cervix, colposcopy, directed biopsy or diagnostic conization. The objective is to exclude the presence of invasive carcinoma and to determine the degree and extent of any cervical intraepithelial neoplasia (CIN). The treatment of CIN includes ablation, cryotherapy and excisional methods like LEEP (loop electrosurgical excision procedure). Evidence from controlled trials show that these techniques are of equal efficacy, averaging 90% success rates in the treatment of CIN.

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LEARNING POINTS

- **The correct sampling of a cervical smear with appropriate equipment contributes to a significant extent to the diagnostic value of the Pap test.**
 - **Cytology identifies women with CIN. At least 90% of these lesions can be removed under local anaesthesia. Removal of CIN can more or less guarantee that the individual woman will not die from cervical cancer.**
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