Pap Smear Collection and Preparation: Key Points

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ABSTRACT

Cytology is the science of study of cells. It is derived from the Greek word “cytos” which means cells. The cells of the cervicovaginal epithelium are continuously evolving. The mature cells reach the surface and are then exfoliated. Initially, these exfoliated cells were collected from the posterior fornix, which showed cells from endocervix, ectocervix, and the vaginal epithelium. Hence, it was known as the exfoliative vaginal cytology. But now, the cells are taken directly by scraping the ecto and the endocervix. A variety of sampling devices are available in the market. The basic aim is to augment sampling of the complete transformation zone (TZ) as well as the squamocolumnar junction (SCJ) and cause least possible trauma to the cervical and endocervical epithelium during its use. The SCJ is of crucial significance for cervical cancer pathogenesis. Most of the precancerous changes take place within the TZ and at the SCJ. Hence, the collection of cells from this area is of utmost importance. The reliability of cervical cytology for the detection of precancerous lesions also strongly depends on immediate wet fixation of the smear. Therefore, the gynecologists or the paramedics who perform the conventional Pap smear must not only be trained in the art of cell collection and smearing of the material onto the glass slides but also learn immediate wet fixation of the cervical cells. Liquid-based preparations have made all these steps relatively easy for them as the design of the Cervex brush is such that it mostly ensures the sampling of the complete TZ and the SCJ. Pre-fixation of cells occurs in the vial containing a weak fixative and the transfer of cells onto the glass slide is standardized by the automated stations designed for this purpose. This chapter gives an in depth description of the prerequisites and precautions while collecting and preparing a Pap smear with different devices, especially for settings where conventional smears are still the norm. Instructions for women undergoing Pap smear and the medical personnel who conduct this test are also highlighted.

Keywords: Conventional Pap smear -collection and preparation, Do’s and don'ts for women undergoing Pap smear, Tips of Pap smear collection for medical personnel, Limitations of Ayer’s spatula

This Chapter Includes:

- Brief anatomy of the female genital tract
- Pathophysiology of the transformation zone (TZ) and the squamocolumnar junction (SCJ)
- Types of cervicovaginal smears
- Collection devices
- Prerequisites and precautions while collecting a Pap smear
  - Basic equipment required for Pap smear collection
  - Do’s and do not for women undergoing Pap smear
  - Do’s and do not of Pap smear collection for the medical personnel
- Technique of collecting a Pap smear
- Tips for gynecologists.

INTRODUCTION

The “Pap test saves lives.” In 1940’s, Dr. G. N. Papanicolaou first developed the technique of collecting, fixation, and staining of cervical cells (Pap smear). Today, it has become a revolutionary technique and a method of choice for the diagnosis of precancerous and cancerous changes in the uterine cervix. No other test has been as successful in stamping out cervical cancer, through the detection of treatable precursor lesions, provided screening is done regularly. A long window of opportunity exists during which regular screening, detection, diagnosis, and management of precursor lesions leads to prevention of invasive cancers and related deaths.

The Pap smear can be used as a screening test for:
- Population screening by organizing camps in urban and rural areas.
- Opportunistic screening method – meaning that all eligible women visiting any hospital or any gynecology outpatient department for any other reason undergoes a Pap smear.
- Pap test is included in regular corporate/non-corporate health checkups.

The first two are more commonly adopted in developing countries where organized screening programs do not exist.

Screening using a Pap test – either conventional or liquid-based cytology (LBC) has stood the test of time. A systematically collected smear from the SCJ, well fixed, and well stained is still the best method to detect abnormalities in the cervical epithelium preceding invasive cancer. These abnormalities can be then confirmed through a directed biopsy, collected during colposcopy, and later ablated either by cryotherapy or loop electrosurgical excision procedure/large loop excision of the TZ.

BRIEF ANATOMY OF THE FEMALE GENITAL TRACT

The female genital tract is composed of the vulva, the vagina, the uterus, the fallopian tubes, and the ovaries [Figure 1]. The fallopian tubes, the uterus, and the upper two-third of the vagina develop from two embryonal structures, the Mullerian (paramesonephric) ducts. The caudal portion of the two Mullerian ducts fuses to form the uterus and proximal vagina. The upper part however remains separated to form the fallopian tubes. Imperfect fusion of the ducts results in formation of vagina septus and uterus septus. The fused ducts contact the urogenital sinus forming the lower vagina and vestibule.

The vulva

The vulva is the external portal of entry to the female genital tract. It comprises labia majora and minora, both covered with keratinizing squamous epithelium. The outer surface of labia majora is hair bearing and inner surface contains numerous sebaceous and apocrine glands. The outer surfaces
of the labia minora have sebaceous glands, whereas the inner surfaces blend with the vagina. The labia show pigmentation from the age of puberty, diminishing on the inner aspect of the labia minora where only a thin layer of keratin is present. This epithelium extends to cover the vestibule as far as the hymen. Located anteriorly between the labia minora is the female counterpart of the penis, the clitoris, provided with a retractile, prepuce-like structure. Located about 1 cm behind the clitoris is the opening of the urethra, the terminal portion of the urinary tract. The lymphatic drainage of the vulva is to the inguinal lymph nodes, which are the primary site of metastases in malignant tumors of the vulva.

The vagina

The vagina opens into the vestibule of the vulva. Mucin secretting glands are present on either side of the vaginal

![Figure 1: Anatomy of female genital tract and the location of cervical cancer.](image)

![Figure 2: (a) Cervical changes after puberty (ectropion) (a1) and histopathology of normal squamocolumnar junction (SCJ) (H and E) (a2). (b) Islands of metaplastic changes within the ectropion in cervix leads to formation of new SCJ (b1). Histopathology showing metaplastic squamous epithelium overlying the glands (H and E) (b2). (c) Macroscopically rounded nodules on ectocervix (blue arrows), which are distended mucous glands (nabothian follicles). Black arrow is showing the new SCJ after the healing of ectopy (c1). Histopathology showing the distended mucous glands (blue arrow) (H and E) (c2). (d) Entry of human papillomavirus through breach in the squamous epithelium. (e and f) Position of SCJ and transformation zone before and after menopause, respectively.](image)
introitus, including Bartholin’s glands, which are situated in the lower vaginal wall, providing protection and lubrication. The vagina is lined by non-keratinized squamous epithelium. It is hormonally sensitive undergoing cyclic changes in reproductive age group and atrophies with menopause.

The cervix

The cervix is a cylindrical structure of approximately 4 cm in length and 3 cm in diameter. It has outer surface embedded within the vagina called as ectocervix or portio vaginalis visible during vaginal examination. It is covered by stratified squamous epithelium. The rest of it is continuous with the body of uterus. The parts of cervix include ectocervix, endocervix (the canal inside the cervix), external os (opening of endocervix in the vagina), and the internal os (opening of endocervix within the uterus). The endocervix is lined by simple columnar glandular epithelium. The protrusion of the cervix into the vagina creates folds of tissues known as fornices with one anterior, two lateral, and one posterior fornices. Exfoliated cells tend to pool in the secretions collected in these fornices. Inside the endocervix deep infoldings of the mucosa called as plicae palmatae travel up to the stroma forming crypts that look like branching glands. The canal is narrow measuring only 2–3 mm and often filled with mucus plug the physical properties of which changes with the ovulation timing.

PATHOPHYSIOLOGY OF THE TZ AND THE SCJ– [Figure 2]

The SCJ is the point at which the cervical squamous and glandular epithelium meet. The location of the junction is more dependent on the age of the patient. Before puberty, the junction is normally at the external os. After puberty, the cervix changes its shape and the endocervical mucosa is exposed on the portio vaginalis, where it is visible as a red zone known as ectropion or eversion. It is sometimes mistakenly called an erosion, although no actual ulceration is present and the condition is essentially physiological [Figure 2a1 and a2, b1 and b2, c1 and c2].

Healing of the exposed endocervical epithelium (ectopy) occurs under the influence of acidic pH of the vagina. The glandular epithelium undergoes metaplastic change to squamous epithelium by differentiation of the reserve cells to a more sturdy squamous epithelium leading to the formation of a new SCJ [Figure 2b1 and b2]. This can also be due to recurring infections and repeated trauma of parturition.

The SCJ is of crucial significance for cervical cancer pathogenesis. The area between the native SCJ/or previous SCJ and the newly formed SCJ is known as the TZ. The reparative/regenerative changes that take place within the TZ and especially at the newly formed SCJ make this region prone to not only infections but also to the entry for the human papillomavirus [Figure 2c1 and c2]. Most of the precancerous and cancerous changes take place within the TZ and at the SCJ. Hence, the collection of cells from this area is of crucial importance. Glands with orifices obstructed by the metaplastic process are prone to distend with mucus, even becoming visible macroscopically as rounded nodules on the ectocervix, known as nabothian follicles [Figure 2d].

After the menopause, the cervix shrinks and the SCJ recedes higher up into the endocervical canal [Figure 2e and f]. The reliability of cervical cytology for the detection of precancerous lesions strongly depends on the proper sampling of the TZ and SCJ, immediate wet fixation, and staining of the smear.

Figure 3: (a) Cotton swabs for collection of smear from the transformation zone, (b) Ayer’s spatula, (c) Szalay’s spatula of different sizes. (d) Cytobrush, (e) Cervex brush.
TYPES OF CERVICOVAGINAL SMEARS

1. Pancervical smear: This is the most common and widely accepted method of collection. In this, cells are collected from the complete SCJ and the adjacent TZ. However, fallacies remain in the adequate sampling of cervices of:
   a. Postmenopausal women (in whom the SCJ recedes higher up in the canal) [Figure 2f]
   b. Nulliparous women with pinpoint external os
   c. Patients who have undergone lower segment cesarean section (LSCS) and thereby have a pinpoint external os
   d. And when endocervical brush alone is used as a sampler. In the latter case, the brush may not collect cells from the SCJ and TZ

2. Posterior vaginal pool smear: This helps in studying cells rolling down the entire female genital tract including cells from ovarian tumors

3. Lateral vaginal wall smear: It is collected from the junction of upper one-third and lower two-thirds of vagina. It is recommended for hormonal assessment

4. Endocervical smear: It is collected from the endocervical canal only

5. Vaginal vault smear: It is collected from patients who have undergone hysterectomy

6. Blind vaginal smear: It is collected from nulliparous, virgins, or patients having stenosed vagina

7. Endometrial aspiration smear: It is collected from patients complaining of postmenopausal bleeding or dysfunctional uterine bleeding (DUB) or suspected of having endometrial carcinoma.

COLLECTION DEVICES [Figure 3]<sup>[1]</sup>

A host of collection devices is available for collection of cells from the cervix, for example, the Ayer's spatula, the Szalay plastic spatulas of different sizes, cotton swabs are also used at some centers and cytobrushes and brooms of different varieties and sizes are available in the market. Any one of these may be used as per the personal choice of the gynecologists, requirement of that particular cervical lesion, and also the cost and availability of the device. The basic aim is to augment sampling of the complete TZ as well as the SCJ and cause least possible trauma to the cervical and endocervical epithelium during its use. However, each of these devices has its own advantage and disadvantage.

1. Cotton swab [Figure 3a]: The applicator sticks are thin bamboo sticks, approximately 12 inches long
   - A moist cotton swab (preferably non-absorbable cotton) should be used
   - If cotton swab is too wet, the cells in smear acquire an exploded appearance and
   - when too dry, the cells are mechanically damaged by the friction between dry cotton swab and glass slide during smear preparation.

   **Advantages and disadvantages of cotton swab:**
   - Advantage: Non-traumatic to the cervical epithelium and is cost effective.
   - Disadvantage: Mucus and moisture are sucked if the cotton swab is dry resulting in poor nuclear images in the smear.

2. Ayer's spatula [Figure 3b]: It is a bifid wooden or plastic spatula with one end longer than the other. It is the most commonly used device. Similar such device is Szalay's spatula [Figure 3c].

   **Advantages and disadvantages of Ayer's spatula:**
   - Advantage: It obtains adequate cellular material from the endocervix, SCJ, and adjacent TZ.
Disadvantage: Occasionally traumatic to the cervical/endocervical epithelium and also if the tip is not fit properly into the external os of the cervix, it may fail to scrape cells from TZ. Causes bleeding when cervix is inflamed.

- Endocervical brushes: It includes cytobrush and Cervex brush.
  a. Cytobrush [Figure 3d]: It is used for sampling of cells from the endocervical canal. It has a narrow tip covered with delicate bristles and is easy to introduce in the endocervical canal
  b. Cervex brush [Figure 3e]: It is a broom-like device composed of soft plastic filaments. The central bristles of the broom are inserted into endocervical canal whereas lateral bristles bend against the ectocervix.

**Advantages and disadvantages of Cervex brush**

Advantage: Cervex brush helps to sample TZ and endocervical cells simultaneously and is used for collection of cervical cells for LBC.

Disadvantage: Sometimes may cause epithelial trauma and bleeding and is costly.

### PREREQUISITES AND PRECAUTIONS WHILE COLLECTING A PAP SMEAR

**Basic equipment required for Pap smear collection [Figure 4]**

1. Sterilized dry bivalve Cusco speculum
2. Clean glass slides labeled with a diamond pencil
3. Sterilized cotton tipped applicator/sticks/Ayres spatula or a Cervex brush
4. A wide mouth specimen bottle with fixative (absolute alcohol) or commercial instant spray fixative
5. Normal saline
6. Glass marking diamond pencil
7. Hanging drop slide and 22 × 22 mm glass coverslips to check for Trichomonas vaginalis
8. Curved metal cannula or a disposable plastic endometrial aspirator (in case of endometrial cytology).

**Do's and do not for women undergoing Pap smear:**

**Instruct the women**

1. To come during the postmenstrual period or best between the 14th and 28th day of cycle (premenstrual period)
2. Not to come when there is active bleeding. First 3–4 days of menstruation are best avoided although not a contraindication
3. To avoid intercourse on the previous day as the contamination with semen material like sperms [Figure 5] can obscure squamous cell morphology and large seminal vesicle cells can mimic atypical squamous cells, as these cells are large and bizarre looking with large polypoid smudged nuclei and almost black chromatin. The clue to identify these cells is presence of cytoplasmic golden brown lipochrome pigment and accompanying sperms
4. To avoid douching with any antiseptic solution
5. To avoid use of pessary

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**Figure 6:** Conventional Pap smear – Glove powder may resemble koilocytes (red arrow) (×40).

**Figure 7:** Immediate wet fixation of slide in a Coplin jar containing 95% absolute alcohol is the most crucial step.

**Figure 8:** (a) Inflamed cervix with purulent discharge on the surface, (b) same cervix after treatment of infection showing a well-demarcated squamocolumnar junction.
Kamal: Key points of Pap smear collection and preparation

6. Not to insert any vaginal tablets as a part of ongoing treatment (need to be withheld or smear collection is done after completion of treatment)

7. Not to come if a per vaginal examination is done in the past 24 h

8. Or a cervical biopsy or cryotherapy or any other procedure has been done in the past 6 weeks. Regeneration atypia in squamous cells can be a cause of overinterpretation of epithelial abnormalities.

Do’s and do not for the medical personnel engaged in Pap smear collection

1. In addition to sociodemographic details, the cytology requisition form must include
   a. Contact details such as mobile number and address
   b. Age of the patient
   c. Clinical history in short like parity, last child birth, biopsy, previous cytology or histopathology reports, and colposcopy findings (if any)
   d. Date of last menstrual period and menstrual history
   e. History of contraception such as use of intrauterine device, oral pills, and barrier contraceptives
   f. Per speculum and per vaginal findings
   g. Exact anatomical site of smear and type of device used

2. Do not use lubricated Speculum. The lubricant jelly can cause artifacts in the smear

3. Smear is collected as the first step after inserting and positioning of the speculum and before any vaginal examination is done. The glove powder can come as
contaminant in the smears and may mimic abnormal cells like koilocytes [Figure 6]
4. Use spatula of suitable size and shape as per the size and shape of the cervix.
5. Smear should not be collected immediately after curettage or biopsy
6. The glass slide, speculum, and spatula should be absolutely dry as the presence of water or moisture on these destroys the cells and hampers cellular details
7. Immediate wet fixation is mandatory [Figure 7]
8. Slides must be fixed, that is, dropped immediately in the bottle containing the fixative to avoid air drying artifacts in the smear. Partial or complete air drying of cells because of delay in fixation leads to poor staining of nucleus and the cytoplasm. A bottle of absolute alcohol needs to be kept ready or LBC vial has to be ready at hand so that there is no delay in immersing the slide in alcohol or brush in the vial respectively
9. If for any reason there is delay in fixation of the smear, do not put it in alcohol. Instead it is safe to allow the Slide to DRY COMPLETELY and sent to the laboratory (wrapped in a paper). This also avoids the hurry and worry of immediate wet fixation that sometimes leads to the spillage of alcohol from jars. [2]

Figure 14: Spray fixation. Spray the slide before it gets dry. Note the angle and the distance of the spray.

Figure 15: Colposcopic appearance of Inflamed and angry looking cervix after application of 5% acetic acid. The smears from such a cervix show partial or totally obscuring blood and inflammatory cells.

Figure 16: Liquid-based cytology preparation: The atrophic smear at times shows less number of squamous cells and significant amount of granular cytolytic material in the background. This is because of dryness of the squamous epithelium.

Figure 17: Liquid-based cytology preparation: Smear from prolapsed cervix, showing scanty cellularity and anucleate squames mostly singly and in compact clusters.
10. Smears should be uniformly thin and the material collected should be evenly distributed.
11. Avoid smearing purulent material alone on the slide [Figure 8a and b]. Too many inflammatory cells obscure morphologic details of epithelial cells and also result in altered staining of the squamous cells [Figure 9a].

Note: Leukocytes are however present even in normal Pap smears [Figure 9b]. The healthy white discharge comprises of leukocytes and is seen in the conventional Pap smear in a linear or circular fashion (used to prepare the smear). The background shows squamous cells free of polymorphs. The polymorphs are healthy and do not show degenerative changes like cytoplasmic vacuolation that is seen in an inflammatory smear.

TECHNIQUE OF COLLECTING A PAP SMEAR [Figure 10][3]

1. Explain the procedure to the patient and take a verbal or informed consent.
2. The patient is instructed to sleep in a lithotomy position – ideally on a PV table.
3. A Sim’s speculum or a bivalve Cusco’s speculum is inserted in the vagina and the cervix is exposed with gentle maneuvering of the speculum. Use of Cusco speculum makes one “hands free.”
4. The long end of the tip of any of the spatulas mentioned above or the tip of the brush is put in the endocervical canal and fixed gently but firmly and then rotated clockwise through 360° 2–3 times [Figure 11a and b] and 5 times if the Cervex brush of Surepath [Figure 11c] is used. With the cytobrush and Cervex brush – reverse rotation during the procedure is strictly prohibited as it may leave behind those cells that are collected in the previous rotations.
5. The material is spread in a circular or linear fashion in the central 2/3rd area of the glass slide leaving the edges of the glass slide free of material [Figure 12a and b].
6. For LBC preparation, the Cervex brush is broken away or detached from the stick and tip of the Cervex brush is dropped in the LBC vial containing fixative [Figure 13a and b].
7. The conventional smear is immediately fixed in absolute alcohol. Delay in fixation causes air drying artifacts and there is clouding of morphological details.
8. However, if there is shortage of alcohol or constant failure to provide “good quality wet fixed smears,” then the smear may be totally air dried. The air dried smears are rehydrated in the laboratory.
9. For spray fixing of the slide, spray should be kept at an angle of 45° and at a distance of 6 inches from the slide [Figure 14].

The requisition form must mention the mode of preservation of the slide as the steps in the processing of the smear in the laboratory are different depending on the mode of fixation of the smear.

- If fixed in absolute alcohol – Usual staining steps are initiated.
- If the slide is sent dry – rehydration with glycerol or normal saline of smear followed by fixation in absolute alcohol needs to be done before staining is started.
- If the slide is fixed with a Spray fixative – before the staining procedure starts, the wax used in spray needs to be removed using an additional step of alcohol.

Tips for the gynecologists

The clinician or the paramedical personnel are the best judge as to whether or not the cervix can be scraped, for example, when it is highly inflamed and angry looking or covered with copious purulent discharge as seen in Figure 8, it is better to call the patient 6 weeks after treatment with antibiotics. Repair of the epithelium leads to clear visualization of the SCJ and TZ and there are less chances that the smear will be unsatisfactory for evaluation due to obscuring inflammation or blood and less number of squamous cells. However, a fair judgment needs to be taken that the patient will return after a course of antibiotics, lest we may lose the chance of screening a high-risk patient.

Similarly when attempting to collect smear from a post-menopausal woman [Figure 16], or from a patient of prolapse uterus [Figure 17], if the cervix is dry and the epithelium is thin and parchment like [Figure 18], scanty material is scraped and transferred on the glass slide. In such a situation,
local application of estrogen cream is advised for at least 3 weeks before taking a repeat smear. This enhances both – the maturation of the epithelium and exfoliation of cells.[4]

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ABBREVIATIONS (In alphabetic order)

DUB - Dysfunctional Uterine bleeding
LBC - Liquid based Cytology
LSCS - Lower segment cesarean section
PV - Per vaginum
SCJ - Squamocolumnar junction
TZ - Transformation zone

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