Cytopathology
**Definition:**

- Cytopathology is the study of normal and abnormal exfoliated cells in fluids.

**Types of exfoliated Cytopathology:**

1. Natural spontaneous exfoliation
2. Exudates and transudate
3. Artificial enhanced exfoliation
1-Natural spontaneous exfoliation

• Natural covering epithelium:
  – skin, urinary tract, vagina, and cervix.

• Glandular epithelial secretion:
  – Breast (Nipple secretion).

• Sputum

• Urine
2-Exudates and transudate

- Pleural fluid = Lung fluid
- Peritoneal fluid = Ascites fluid
- Pericardial fluid = Heart
- Synovial fluid = Joint fluid
- CSF
3-Artificial enhanced exfoliation

- Scrapings from cervix, vagina, oral cavity, and skin.
- Brushing and lavage: bronchi, GIT, and urinary tract.
- Fine needle aspiration (FNA) for:
  - Body cavity fluid: pleural, pericardial & peritoneal fluids,
  - Cysts: neck, breast & ovary,
  - Solid tissue: body organs, tumors & other as well.
ROLE OF CYTOPATHOLOGY

• Early detection of unsuspected diseases (malignant or pre-malignant lesions).
• Confirmation of suspected diseases without surgical trauma.
• Diagnosis of hormonal imbalance.
• Useful in flow up the course of disease or monitoring therapy.
Quality Control
**Quality control issue:**

1. Age or birth date
2. LMP, if pre-menopausal
3. Date of specimen collection

**Smear screening:**

Smears can be screened using various strategies. The horizontal Z-sweep technique has proven to be effective. Smears are to be screen using at least 10x magnification.

Results of cases that have been selected for screening will not be reported until the screening process is completed.
*Satisfactory for evaluations:*
*Appropriate labeling.
*Relevant clinical information.
*Adequate numbers of well preserved and well visualized squamous epithelial cells spread over more than 10% of the slide surface.

Fixation or preservation is one of the most important steps in the procedure. Drying of the cells prior to fixation will usually result in artifacts such as nuclear distortion and vacuolization.

*Satisfactory for evaluation but limited by:
This does not necessarily require a repeat specimen. The term used if any of the following:
*Lack of pertinent clinical information.
*Partial obscuring blood or inflammation.
*Partial thick specimen areas.
*Partial poor fixation.
*Partial air drying artifacts.
*Lack of endocervical/transformation zone component.

(Within normal limits), maybe used without further explanation, can be signed out by the cytotechnologist.
*Unsatisfactory for evaluation:
This indicates that the specimen is unreliable for the detection of **cervical epithelial abnormalities**.

It is used if any of the categorization criteria apply:
- Lack of patient ID on the specimen and/or requisition form.
- Technical unacceptable slides: broken, cannot be repaired, or if cellular material is inadequately preserved.
- Scant squamous epithelial spread over less than 10% of the slide surface.
- Completely obscuring blood or inflammation.
- Thick specimen areas.
- Poor fixation.
- Air drying artifacts.

If **abnormal cells** are present, a specimen cannot be called unsatisfactory. The diagnosis is based on the abnormal cell findings; the adequacy is evaluated as satisfactory or satisfactory but limited.
*Principle (staining protocols, Reagent).

• Optimal staining is a fundamental prerequisite in cytology. Accurate identification features depend on the recognition of appropriate stained cellular entities and the diagnostic confidence is based on the well stained and technically controlled cytopreparations. Therefore, stain characteristic, stain intensity and overall quality must be reproducible and permanent.

• In this lab, the progressive papanicoloau staining method is useful for fixed cytopreparations and the Diff-Quick staining method for Air-Dried staining.
**Quality control issue:**

- The staining quality of gynecological, non-gyn and FNA cytopreparations is monitored daily and documented in the technical QC/QA.

- The overall (technical, staining and reporting) quality of gynecological, non-gyn and FNA cytopreparations is monitored and documented by the pathologist reviewing cases utilizing a QC feedback system.

- All reagents/stain dishes must be maintained clean, free of reagent or cellular contamination, covered when not used, and be properly labeled, dated as deemed appropriate.

- Whatman filter paper is recommended for filtering suspended contaminants or cells from reagent/stain solutions.

- **All staffs** are required to understand the safety issues that apply when handling the reagents used in cytology as outlined in Internal Policy Procedure, includes review of the laboratory safety manual.
The Internal Policy Procedure (IPP) outlines:

- Progressive Papanicolaou staining
- Leukostats Diff-Quick staining
- H &E staining
- Decolorizing slides for re-staining
- Reagent contamination control
- Reagent receipt, labeling, preparation, usage, disposable and storage.
- Overall quality control procedures
Reagent receipt and labeling, preparation, usage, disposal and storage:

• The safety issues regarding the reagents in cytology in detail. Every attempt is to be taken to ensure that all reagents are used properly to alert all staff of their identity.

• Once received, all reagent containers are labeled as follows:
  – Date of receipt
  – Expiration date

• Reagent usage, every attempt must be made to utilize reagents within published expiration dates. Once opened, all reagent containers are labeled by the date of opening.

• Reagent disposal, the general handling and disposal of biological infectious and of chemicals/reagents is described in the laboratory safety manual.

• Reagent storage, the general handling, disposal and storage of biological infectious waste and chemicals/reagents is described in the lab safety.
Coverslip quality control:

• **Coverslipping** is the fundamental aspect of technical cytology. The purpose of a coverslip is to cover, isolate, preserve and protect stained cellular material and to provide transparent surface through which may be examined under microscope. The coverslip is cemented to the slide with a mounting medium.

• To eliminate cellular cross contamination of slides or mounting medium, the transfer pipette that is used to transfer mounting medium onto coverslips must never come into contact with cellular material.

• Coverslipped slides must be allowed to dry adequately prior to filing to avoid slide adherence. Adhered slides are difficult to separate.

• Air bubbles formed while coverslipping compromise the technical quality of slides. No air bubbles should remain.
**Coverslippping procedure:**

- For fixed slides:
  - Wear protective gloves, work on clean, flat surface covered with towel
  - Coverslips should be clean, dust free.
  - Place a drop of mounting medium on the coverslip, remove the slide from xylene and wipe excess xylene present under the slide with clean tissue or gauze.
  - Gently place the slide (cellular material) over the coverslip at an angle, to allow medium adherence
  - Excess medium that may ooze out of the slide edges can be wiped off with a xylene soaked gauze.
  - Do not allow the cells to dry out at any stage.
  - The end result should be a presentable slide with the coverslip centered over the cellular material, with no mountant on the coverslip surface, and no air bubbles.
  - If air bubbles exist or if there is excessive amount of medium, remove the coverslip by soaking the coverslipped slide in xylene until the coverslip loosened. Repeat the procedure
  - Place coverslipped slides on the slide warmer for quick medium hardening.
Coverslipping, recoverslipping:

- The objective is to coverslip the slide evenly to avoid drying-out pockets.
- Recoverslipping maybe performed once the original coverslip removed.
- Older slides with hardened mounting medium require soaking in xylene until the coverslip is removed. A slid warmer is used to quick mounting hardening. The warmer must not be too hot to touch (just warm).
The PAP smear
The PAP smear

- Cervical cancer screening began in the United States in the late 1940s after Dr. George Papanicolaou developed the Pap smear.
- A Pap smear (also called a Pap test) is a screening test used to examine cells from the cervix and the vagina.
• (The cervix is the portion of the uterus that protrudes into the vagina). **Cervical and vaginal cells are studied to determine whether there is evidence of cancer or pre-cancerous changes.**

• If **abnormal cells** are found, they are classified according to their **degree of abnormality**.

• Most abnormal Pap smears are caused by cervical infections or inflammation which can usually be successfully treated before leading to cancer.
• A Pap smear is used to screen a clinically normal appearing cervix for cancer of the cervix or its precursors. When there is an obvious lesion, especially one that is elevated, ulcerated or covered with necrotic exudate, a biopsy is necessary.

• Pap smears of the cervix are inappropriate to screen for other malignancies of the female genital tract, (i.e. a patient with post-menopausal bleeding should have an endometrial biopsy if her cervix is normal).
*Abnormal Pap smear findings may indicate:

- Infection (including the human papilloma virus HPV)
- Swelling or inflammation
- Pre-cancerous cell changes
- Cervical cancer
The Pap Smear test is the only screening test for cancer, in the world, which has caused a decrease in occurrences and deaths from cancer.

A Pap Smear is a screening tool, not a diagnostic test; further evaluation is required when abnormal changes are detected.

A normal Pap Smear is not a guarantee of no cancer; it does not detect cancers of the uterus, fallopian tubes, or ovaries.

Your health care provider may advise you to continue having regular Pap smears even after a hysterectomy.

Your health care provider will determine when you should have your next Pap smear, though every 6 to 12 months is usually recommended.

Pap Smears can be safely performed during pregnancy.
Sample obtaining from the female genital tract are made outside the cytopathology lab, which followed by two main ways of Gynecological smears preparation:

A. Conventional = Traditional glass slide sample technique (Manual way)

B. Liquid-Based Cytology (Automated way)
   1. Thin-prep
   2. SurePath
1. Typically a brush sample is prepared from the endocervix and the spatula sample from the ectocervix.
2. Smears made and fixed immediately after obtaining to preserve the cellular details (spray fixative are suitable).
3. Slides are labeled and placed in a suitable container to prevent specimen contamination or breakage during delivery to the lab.
4. Smears are delivered to cytology lab from nursing unit.
A cervical sample is collected by 2 main ways, either by

1. Using spatula and brush, or
2. Using broom.

In both ways the tip of the collection devise must be rinse in the thin prep solution in the thin prep vial quickly and vigorously at least 10 times and push against the vial wall. The sample vial is capped labeled and sent off to the lab.
Cell enrichment:

The Thin-Prep processor will automatically breaks up blood, mucus, and non-diagnostic debris (gentle dispersion step), and then thoroughly mixes the sample. Then, a filter will collect a thin, even layer of diagnostic cellular material.
Automated cell to slide transfer and automated staining:

A complete automated processing of the samples leading to a uniform thin-layer on the slide. Slide Processor continues the process with Pap stain (EA/OG and Hematoxylin stains).
Sample collection:

A cervical sample is collected with broom. Then, the tip of the collection devise separated from the stem and dropped into sample vial containing ethanol based preservative fluid. The sample vial is capped labeled and sent off to the lab.
Cell enrichment:

By adding density reagent and centrifugation that separate obscuring blood, mucus and other debris from diagnostic material to end with the removal of the debris to create an enriched cellular sample.
Automated cell to slide transfer and automated staining:

A complete automated processing of the samples leading to a uniform thin-layer on the slide. Slide Processor continues the process with Pap stain (EA/OG and Hematoxylin stains).
Comparison between the Conventional smear and the LBC

<table>
<thead>
<tr>
<th>Conventional smear</th>
<th>LBC</th>
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<tbody>
<tr>
<td>Majority of cells not captured</td>
<td>Virtually all of sample is collected</td>
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<tr>
<td>Non-representative transfer</td>
<td>Randomized, representative transfer</td>
</tr>
<tr>
<td>Clumping and overlapping</td>
<td>Even distribution</td>
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<tr>
<td>Obscuring material</td>
<td>Minimizes obscuring material</td>
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Thank You!