



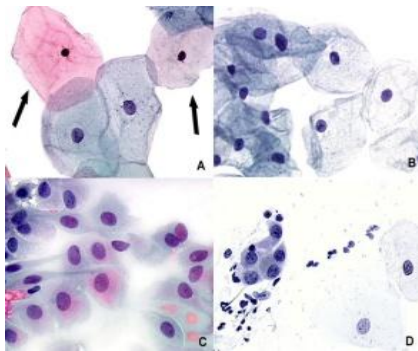
College of Medicine
Second Year (2024-2025)

University of Baghdad

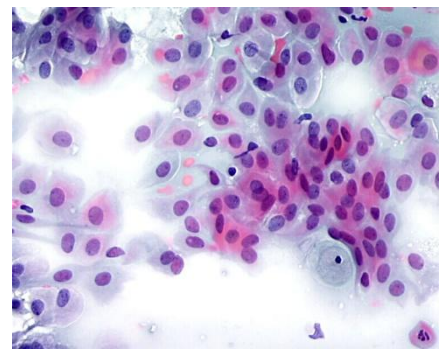
Emeritus Prof. Nada Al Alwan

Diagnostic Cytopathology

Definition: Diagnostic or Clinical Cytology is the science of study and interpretation of normal and disease-altered cells obtained from various sites of the body (i.e., through the detection of abnormal morphologic characteristics of the examined dissociated human cells). George N Papanicolaou introduced cytology as a tool to detect cancerous and pre-cancerous lesions in 1928. Currently, it is a widely accepted method for mass screening in asymptomatic population.



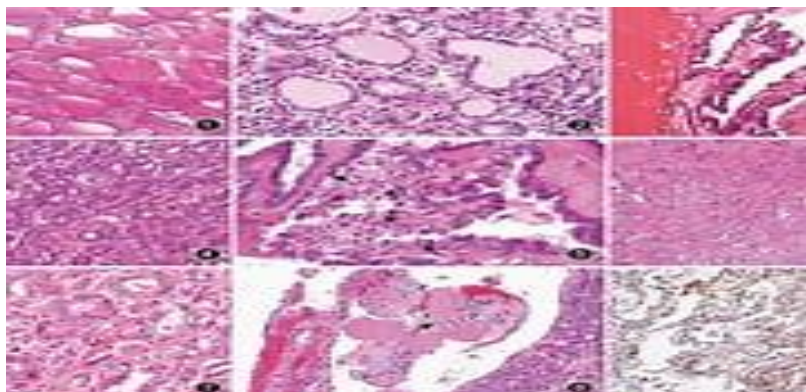
Cytopathology - Cell
Smears
(Papanicolaou Stain)



Cytopathology in relation to Histopathology

In general, Cytopathology has several advantages; being a non-invasive, simple, often painless procedure that is relatively inexpensive and can yield rapid results within even minutes. That is why it has a high population acceptance specifically in the field of screening and early detection of specific cancers.

Histopathology –
Tissue Sections
(H & E Stains)..



These **Advantages of Cytopathology** could be summarized in the following Table:

Histopathology	Cytopathology
<ul style="list-style-type: none"> • Deals with the form and the structure of the tissue. • Evaluation usually begins with a tissue biopsy. • More invasive traumatic procedure is needed; utilizing surgical instrumentation such as forceps, scissors, etc..) • Needles if used should have a large gauge (i.e., Tru-cut needles measuring 14, 16) . • Diagnosis obtained after days. • Basic stain is H&E • Paraffin blocks are needed • Difficult to identify specific causative inflammatory pathogen. 	<ul style="list-style-type: none"> • Deals with the structural changes within the nucleus and cytoplasm of individual cells • Evaluation requires cells only. • Inexpensive simple means of diagnosis which allows frequent repetition of cellular sampling (since it causes no tissue injury). • Fine needles with 22, 23 or 24 gauges are usually used. • Rapid diagnosis that could be obtained within minutes. • Basic stain is Pap stain (however H&E could be used as well) • Mainly slides are needed • Smears permit better evaluation of the nature of the inflammatory process. Fungi and parasites are usually easier to be diagnosed.

Limitations of Cytopathology:

As a rule, histopathology remains the gold standard technique in diagnostic pathology. The whole presented tissue definitely provides a more accurate precise diagnosis of the disease in question. The interpretation of the morphological cellular changes is based mainly on individual observations and often cannot be forced into rigid criteria. Thus, in many cases the cytological diagnosis cannot be final and needs confirmation by histopathology for the following reasons:

- Often the nature of the lesion is not so obvious as in a histological section.
- The exact location of the lesion could be difficult to be pinpointed by cytology: for example squamous cancer cells found in a cytology sputum sample may have originated from the buccal mucosa, pharynx, larynx or bronchi.
- The size of the lesion cannot be approximated by cytology.

- The type of the lesion –e.g., in situ carcinoma as compared with early invasion is more difficult to assess since the relationship of the cells to the surrounding stroma cannot be determined by cytology.

The accuracy of the cytologic examination from any body site depends greatly on the quality of sample collection, preparation, staining and interpretation of the material. Inadequacy in any of these steps will adversely affect the quality of diagnostic cytology.

Cytopathology Sampling Techniques:

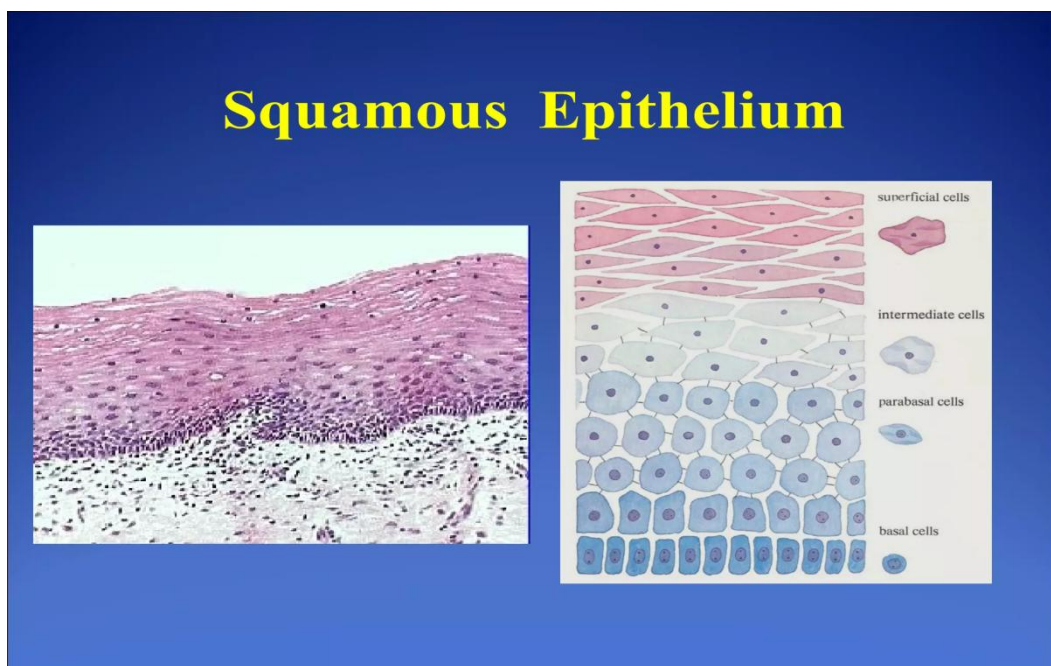
In general, diagnostic Cytopathology is based upon three basic sampling techniques:

1. Collection of **exfoliated cells**.
2. Collection of cells removed by brushing or similar **abrasive techniques**.
3. **Aspiration biopsy** or removal of cells from non-surface –bearing tissue (or deep organs) by means of a needle, with or without a syringe.

1- Exfoliative Cytology

Exfoliative cytology is based on spontaneous shedding of cells derived from the lining of an organ into a cavity, where they can be removed by non abrasive means.

Typical examples are:



- The **Vaginal Smear** which is prepared from cells removed from the posterior fornix of the vagina: The cells that accumulate in the vaginal fornix are derived from several sources that include the squamous epithelium that lines the vagina and the vaginal portio of the uterine cervix, the epithelial lining of the endocervical, and the endometrium. These cells accumulate in the mucoid material and other secretions of the uterus and vagina. In addition to the cells derived from the epithelial linings, the vaginal fornix often contains leukocytes and macrophages that may accumulate there in response to an inflammatory process, and a variety of microorganisms such as viruses, bacteria, fungi and parasites that may inhibit the lower genital tract.
- **Sputum examination:** The sputum is a collection of mucoid material that contains cells derived from the buccal cavity, pharynx, larynx, trachea, bronchial tree and the pulmonary alveoli; as well as inflammatory cells, microorganisms, foreign material , etc.

The same principle applies to:

- **Voided urine**
- **Body fluids**
- **Nipple discharge**

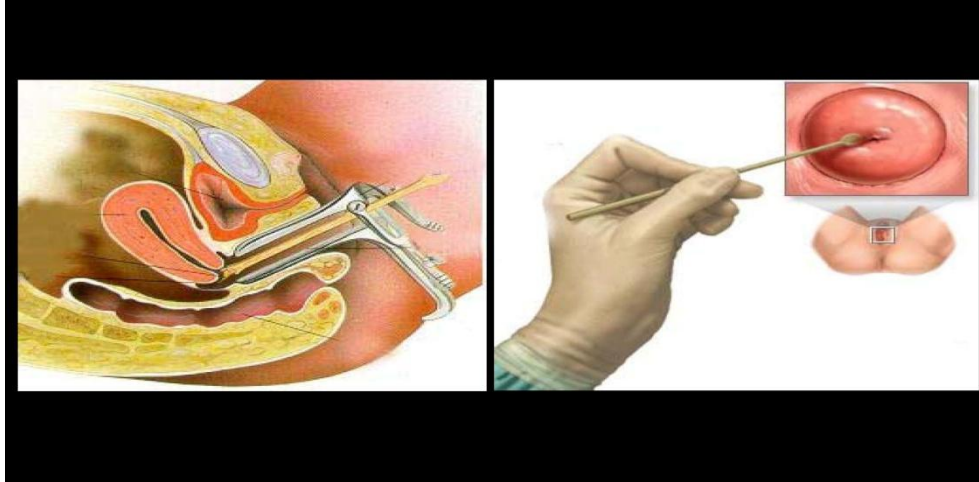
Exfoliative cytology is the simplest of the three sampling techniques. To prevent the deterioration of cells by air-drying or by enzymatic or bacterial actions, the material should be processed immediately without any delay. Alternatively, the use of fixatives, such as alcohol or other cell preservatives, is indicated.

2- Abrasive Cytology

The purpose of this procedure is to enrich the sample with cells obtained directly from the surface of the target of interest. Hence, cell specimens are usually obtained through superficial scraping of the lesion (artificial mechanical desquamation). Examples include:

- **Cervical scraping** or the so called **Pap smear:** the cervical scraper introduced by Ayre in 1947 (i.e., Ayre's Spatula) allows a direct sampling of cells from the squamous epithelium of the cervix and the adjacent endocervical canal.
- **Buccal mucosal smear**

- **Skin scraping** of various lesions
- **Brushing techniques** through using rigid endoscopic and fiber optic instruments to collect cell samples from the gastrointestinal tract, bronchial tree, etc.



The Pap Smear Test

Pap Smear test is a simple procedure in which cells, collected from the cervix, are examined cytologically to exclude abnormal changes. It is used mainly to detect precancerous and cancerous lesions of the uterine cervix. Cervical carcinoma is one of the most preventable cancers; because most of the cellular changes which may lead to carcinoma can be detected and accordingly treated at an early stage before progression. In general, cervical carcinoma develops slowly over a period of up to 10 years; whereby it passes into different pre-neoplastic conditions before it reaches the cancer stage; termed **Dysplasia** or **Cervical Intraepithelial Neoplasia (CIN)**:

Mild Dysplasia ---► Moderate Dysplasia ---► Severe Dysplasia ---►
CIN I CIN II CIN III

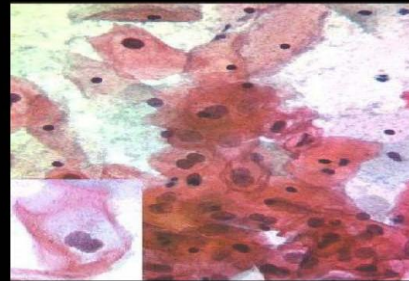
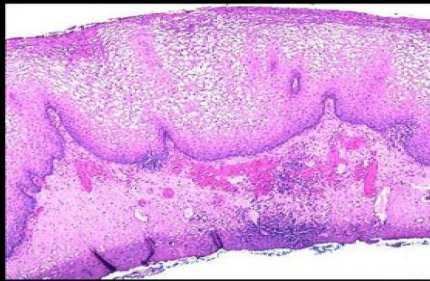
Carcinoma in Situ ---► Carcinoma

Microscopical examination of the exfoliated cells from the uterine cervix through Pap Smear test should therefore lead to the detection of these pre-neoplastic lesions in their earliest stages (i.e., stages of mild dysplasia). Accordingly, giving the appropriate treatment would eventually prevent cervical cancer. No anesthesia is required, and the diagnosis could be obtained within few minutes. The procedure

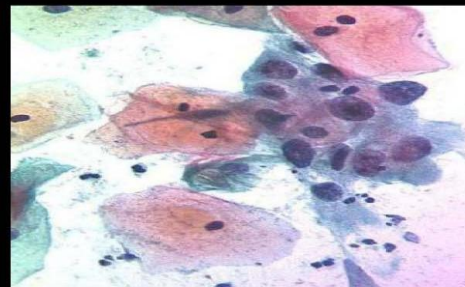
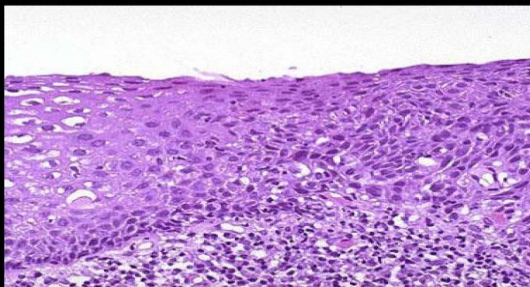
could be easily carried out in any clinic or specialized laboratory. It is recommended that all women between the ages of 18 and 70 years, who have ever had sexual activity, to have the test every 2 years.

Pap smear could also allow the diagnosis of other microbiological agents such as *Monilia*, *Trichomonas Vaginalis*, Herpes Simplex or **Human Papillomavirus “HPV”**. In the latter condition, the detection of "*Koliocytotic atypia*" within the squamous cells, could signify the diagnosis of HPV and hence recommends urgent follow up – as that might cause progression of dysplasias to carcinoma when untreated.

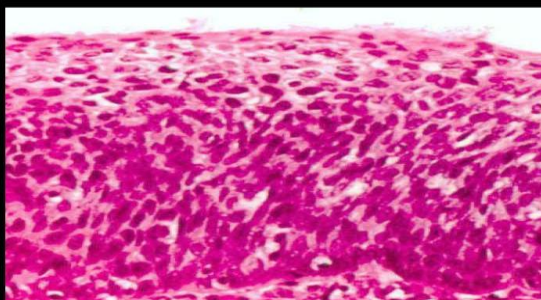
HPV – Mild dysplasia



Moderate dysplasia



Severe dysplasia



"The Bethesda Classification System" for nomenclature of Dysplastic Lesions:

Squamous & Glandular abnormalities

The term Suamous Intraepithelial Lesions (SIL) encompasses the spectrum of the precursors to invasive squamous cell carcinoma (i.e., dysplasia, carcinoma in situ and CIN). On the other hand, changes in the glandular cells are termed: **Glandular Intraepithelial Lesions (GIL)**.

Grading of SIL (Squamous Intraepithelial Lesions)

The Bethesda System recommends a Low-grade / High grade approach in classifying SIL. Most LSILs are secondary to transient infections that carry little risk of transition into carcinoma, whereas most HSILs are associated with viral persistence and a significant potential for progression to invasive cancer.

Low- grade squamous intraepithelial lesions (LSIL): Encompass lesions of "mild dysplasia" (CIN 1) and "koilocytosis" (signifying Human Papilloma Virus infection).

High- grade squamous intraepithelial lesions (HSIL): Comprise lesions of "moderate dysplasia" (CIN 2) and "severe dysplasia" (CIN 3).

3- Fine Needle Aspiration Cytology (FNAC)

In general, the definitive diagnosis of any mass can be established by:

- ✓ Open biopsy
- ✓ Tissue core needle (Tru-cut) biopsy
- ✓ Fine needle aspiration biopsy.

Compared to FNA, Tru-cut biopsy is a more traumatic procedure which should be performed under local anesthesia. It requires more time and special equipment that are more expensive. Pain, discomfort and bleeding are common complications.

FNAC, on the other hand, provides many advantages to the surgeons being an easy, reliable, cost effective diagnostic technique which could give rapid results. The procedure could be performed in an office setting without anesthesia. It is usually not more painful than a venipuncture and can be repeated immediately if the acquired material is inadequate. Many cancer diagnostic centers included FNA as a feasible test to provide preoperative diagnosis of breast cancer and to reduce the number of operations for benign breast diseases.

When reduced to its simplest terms, FNAC consists of:

- ✓ Using a needle and a syringe to remove material from a mass.
- ✓ Smearing it on a glass slide.
- ✓ Applying a routine stain.
- ✓ Examining it under the microscope.

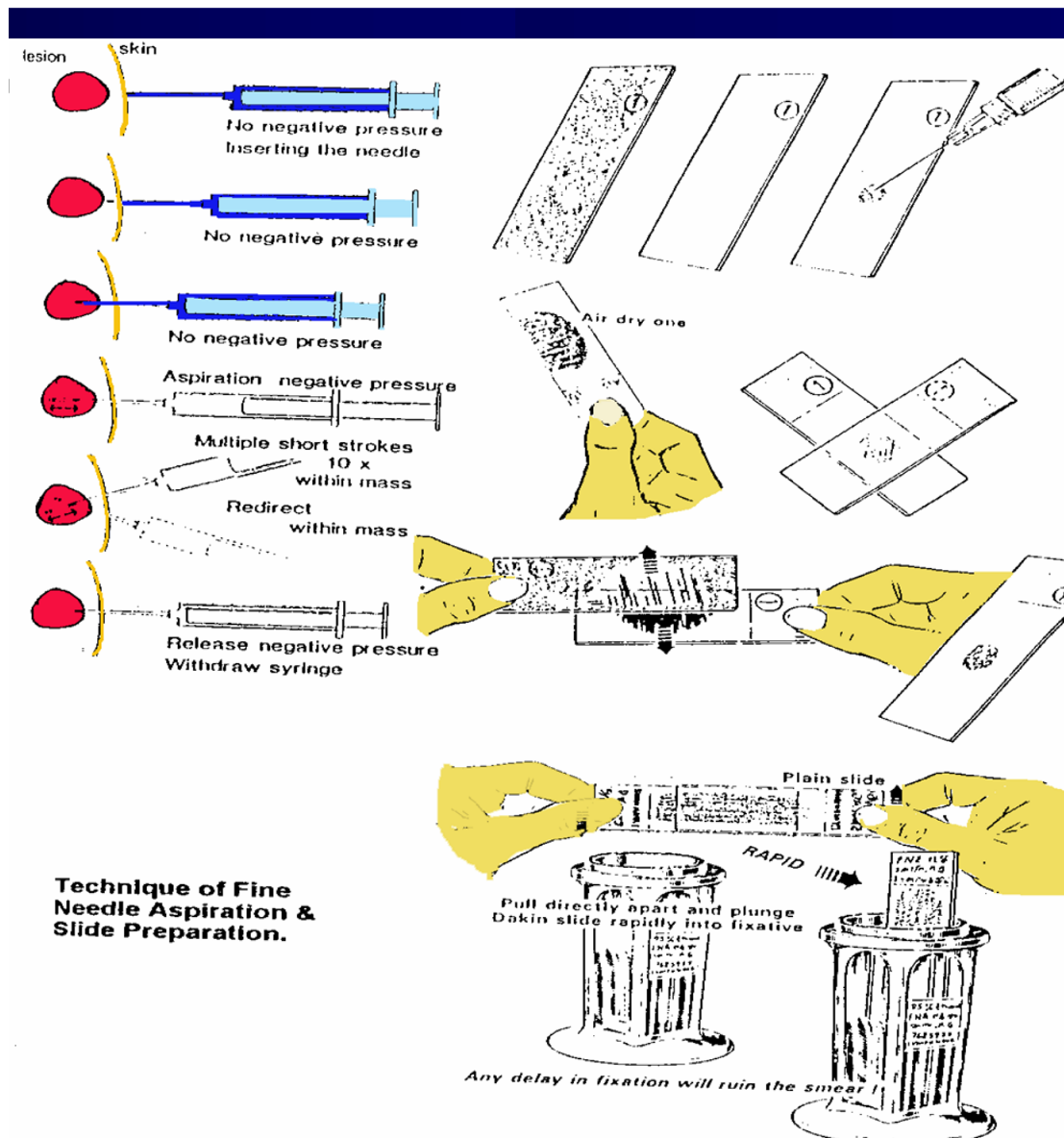
Technique of FNAC:

The skin over the palpable mass (within the breast, lymph node, or any other organ) is sterilized with an antiseptic. Utilizing the index and the middle finger or the thumb and the index finger, the mass is localized with the non-dominant hand. With firm downward pressure on the skin over the mass, it should be compressed against a rib and stabilized.

Aspirates should be obtained using preferably a 23 gauge, 1 ½ inch disposable needle mounted on a 10 ml plastic syringe, held by the dominant hand. Larger needles (22 gauges) are used in aspirating material from hard fibrous masses and in cases of suspected cyst or abscess.

Without using anesthesia, the needle should be gently introduced through the skin passing to the level of the dominant mass. Having confirmed the position of the needle within the mass, negative pressure should be created within the syringe by pulling back the plunger. The needle should move back and forth through the mass, in different rotational directions using sewing-like-motion. Suction should be maintained throughout the process by outward pressure of the right thumb on the underside of the syringe. By this method the needle can cut loose many small pieces of tissue that are then readily aspirated due to the suction applied by syringe. These to and fro strokes should be repeated at least 6-8 times. In general most of the aspirations are terminated when material begins to appear in the needle hub.

The following step should be designed to protect the material that has been obtained. All suction should be released before removing the needle from the mass. This is accomplished by allowing the syringe plunger to return gently to its resting position. Then the needle should be withdrawn gently from the mass. To limit hematoma formation from the site of the puncture, firm pressure should be applied with a piece of cotton for two minutes.



FNAC Procedure

Equipments needed for FNAC procedure:

Syringes

The key feature in a syringe is lightness in weight, adequate in volume, and one hand control. Modern sterile disposable plastic syringes are perfect for use in FNAC. Most practitioners use the 10 cc size, while some prefer the 20 cc size (specifically when a cyst is suspected).

Needles

Several variables should be considered in choosing the proper length and gauge. In general the length should be at least 1.5 inch (3-8 cm). This is desirable in the breast because many lesions are found deeper than expected. It has also been demonstrated that small gauges (22-23) are preferred since they are rigid enough and at the same time cause less sensation and hematoma. Needles with a plastic hub rather than a

metal hub are advisable as this permits the aspirator to monitor the recovery of tissue, blood or other fluids as they appear in the hub.

Glass Slides

Common routine slides measure 75 mm X 25 mm X 1mm. An important procedure is slide labeling at the time of sampling. That is why slides which have one frosted end are preferred. Various substances are used to increase the adherence of specimens to the slide surface, the most commonly used being albumin.

Fixatives

These are applied to the smears as a spray or by immersion of the slide into a liquid. The most commonly used is 95 % Ethanol. This inexpensive readily available liquid provides excellent cytological details. Its only disadvantage is the need to store and transport slides in a container of liquid and to have them immersed in for at least 20 minutes.

Routine Stains

For cytological diagnosis, fixed material is usually stained by **Papanicolaou Stain** (which is preferred) or by **Hematoxylin and Eosin (H&E)** method. Both employ one of the Hematoxylin for staining of the nuclei. This dye colors the nucleic acids as a dark purple blue color. The counter staining with Eosin is designed to show cytoplasmic characteristics. With the Pap stain one hallmark is the use of Orange-G, which stains cytoplasmic keratin with a bright orange color. Air-dried smears on the other hand are usually stained by any **Romanowsky** stains, which are various combinations of methylene blue and its breakdown products (Azure A, B, &C) with Eosin. Most rely on Methanol fixation.

Papanicolaou Stain

All slides should be wet fixed within 95% or absolute Ethanol before staining:

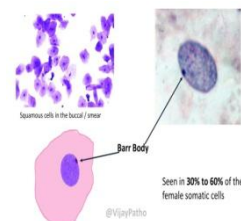
95% ethanol	10 dips
80% ethanol	10 dips
70% ethanol	10 dips
50% ethanol	10 dips
Distilled water	10 dips
Hematoxylin Stain	1-2 min.
Running Tap water	10 dips
50% ethanol	10 dips
70% ethanol	10 dips
80% ethanol	10 dips
95% ethanol	10 dips
Orange-G Stain	2 min.
95% ethanol	10 dips
Eosin Stain	2 min.
95% ethanol	10 dips
95% ethanol	10 dips
Absolute ethanol	10 dips
Absolute ethanol: Xylol (50:50 mixture)	5 min.
Xylol	20 min.
Mount with Canada Balsam	

Pitfalls to Reliable Results from FNAC:

- No mass
- Vague mass
- Mass being too small (less than 1 cm)
- Mass being too deep
- Gross blood into the syringe (i.e., RBCs can mask the picture)
- Scanty cellularity in the aspirated mass
- Dense fibrosis
- Gross fluid

Indications for Cytopathology

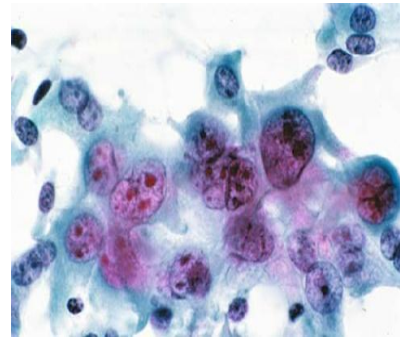
- 1- Differentiation between benign and malignant lesions.
- 2- Diagnosis of malignancy and its type, as well as the identification of the neoplastic cells in primary, metastatic (secondary) or recurrent tumors.
- 3- Diagnosis of premalignant diseases: i.e., detection of precancerous or dysplastic cellular changes; graded as mild, moderate and severe. In the uterine cervix for example, those lesions could be eliminated by treatment of the associated inflammation and causative pathogen or by excision of the lesion.
- 4- Detection of inflammation and certain types of pathogenic agents: i.e., the cytopathologist could prove that the cause of vaginal secretion is vaginitis due to *Trichomonas vaginalis*, Moniliasis or Human Papilloma Virus infection (HPV) through koilocytotic atypia. Similarly the cause of cystitis may be related to bilharziasis (through the detection of Bilharzial ova in smears of urine samples).
- 5- Study of the hormonal patterns and evaluation of the gonadal hormonal activity: through the examination of the squamous cells in vaginal smears; which are under the influence of ovarian hormones.
- 6- Follow-up and monitoring of response to chemotherapy and irradiation; the latter producing certain cellular features which could be diagnostic on cytologic examination.
- 7- The identification of sex chromosome: if a newborn presents with ambiguous genitalia, one cannot tell whether the sex is male or female. The presence of a dark dot attached to the nuclear membrane from inside (Barr body +ve) indicates that a female sex chromosome is present, i.e., the genotype of the baby is XX (♀). Conversely the absence of this Barr body indicates that there is no X chromosome and accordingly the newborn is genotypically a male ((XY ♂).
- 8- Tumour markers study on cytological specimens, i.e., detection of Estrogen, Progesterone or Her2 receptors in breast carcinoma cells.



Cytological Criteria of Malignancy

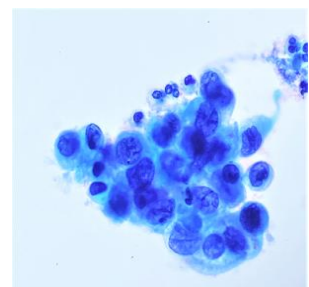
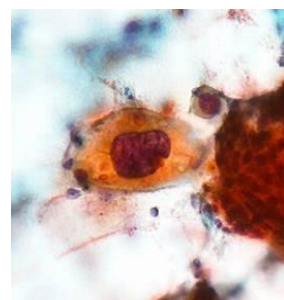
A) Nuclear Changes:

- Nuclear hypertrophy: Nuclear enlargement leading to increased N/C ratio
- Nuclear size variation
- Nuclear shape variation
- Hyperchromatism and chromatin irregularity:
Referring to increased chromatin materials. In malignant cells the chromatin is not evenly distributed within the nucleus; appearing as coarse clumps, in contradistinction to normal cells, which have evenly distributed chromatin.
- Multinucleation: malignant cells may contain more than one nucleus. However, some normal cells such as hepatocytes and histiocytes may contain more than one nucleus. Multinucleated malignant cells differ from nonmalignant multinucleated cells by the fact that the nuclei of malignant cells are unequal in size.
- Irregularity of the nuclear membrane.
- Irregular and prominent nucleoli: giant nucleoli or multiple nucleoli may be present that differ in their sizes and shapes.



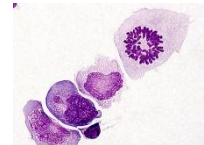
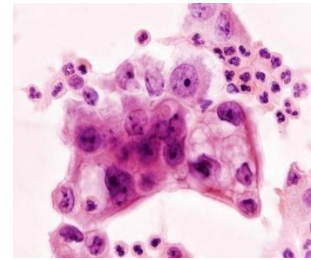
B) Cytoplasmic Changes:

- Scantiness of cytoplasm: in consequence to the high N/C ratio.
- Cytoplasmic boundaries: sharp and distinct in squamous carcinomas and indistinct in undifferentiated carcinomas.
- Variation in size .
- Variation in Shape.
- Cytoplasmic staining: deep orange in keratinizing squamous carcinomas or basophilic in immature poorly differentiated carcinomas.
- Cytoplasmic inclusions: e.g. melanin pigments in melanoma.
- Cytoplasmic and nuclear membrane relationship: cytoplasmic borders of malignant cells could be tightly molded against the nucleus, touching it in more than one place.



C) Changes in Malignant Cells as a Group:

- Cellular phagocytosis or “Cannibalism”: i.e., one cell appearing to be contained in a vacuole of the cytoplasm of another epithelial cell; indicating rapid growth within a narrow cavity.
- Variation in the size and shape of the cell clusters or sheets.
- Lack of cellular adhesion: due to abnormalities in the desmosomes.
- Abnormal mitosis: malignant nuclei may show multiple poles e.g. three poles (tripolar mitosis; triploidy) or four poles (tetrapolar mitosis; tetraploidy). This is abnormal since normal mitosis has two poles (bipolar). Thus normal cells show euploid or diploid pattern on flow or image cytometry whereas malignant cells may show abnormal aneuploid patterns.
- Bloody background: fresh blood is meaningless, but malignancy is suspected when the blood is old, partially ingested by histiocytes or present within a specimen which is obtained without trauma.
- Foreign cellular structures: e.g. psammoma bodies in a routine vaginal smear.
- Degeneration and inflammation as a result of erosion or necrosis within the malignant tumour which could indicate invasion (i.e., tumour diathesis).



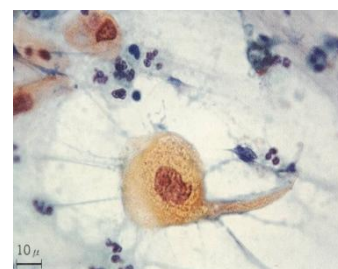
Most Common Types of Malignant Cells:

The best demonstrable tool to observe the various types of malignant cells which could be encountered cytologically is through studying the different variants of “Bronchogenic Carcinoma” through Sputum Cytology.

A routine recommendation for suspected patients is the examination of three successive early morning sputum samples. Histologically, there are four major different types of bronchial cell carcinoma that could be recognized on cytological examinations:

1. Squamous Cell Carcinoma

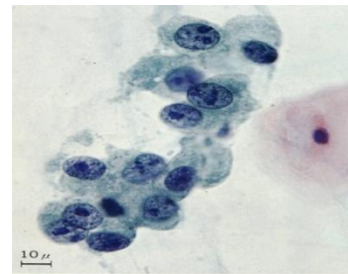
Is the most common and easiest type to be diagnosed by cytologic examination of sputum because of its central position within the bronchial tree..



Cytologically, most malignant cells exfoliate singly but some may be observed in clusters or sheets. Cells often assume variable sizes and shapes; could be amoeboid in shape, assuming pseudopod-like projections. Spindle or tadpole cells could be also detected. The background is usually obscured by necrotic debris, mucous and RBCs (tumour diathesis). The cytoplasm is abundant, dense and granular with a glassy orange appearance in well-differentiated types due to the presence of keratin (stained by Orane G). Anucleated deep orange squames may be evident in long standing cases. The nucleus may show nuclear size and shape variation with irregularity of the nuclear membrane. Marked hyperchromasia is often present; the nuclei appearing very dark or pyknotic. Cannibalism is common. Red prominent nucleoli could be present.

2. AdenoCarcinoma

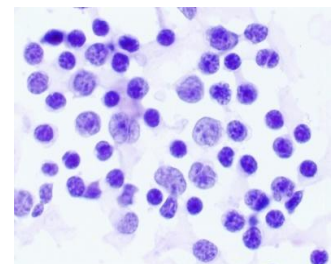
Usually peripheral in position. Cells exfoliate in clusters, sheets or acini; rarely singly. They are often round, oval and irregular in shape. Cytoplasmic vacuoles are observed in well-differentiated types due to the presence of mucous droplets. Large vacuoles may push or compress the nucleus to one side giving an irregular moon-shaped appearance: signet-ring pattern. Neutrophils maybe seen within these vacuoles. Nuclei are often round or oval with moderate hyperchromatism and irregular nuclear membrane. Single or multiple diagnostic nucleoli are usually found. Multinucleatin may occur. Cells stain positive with PAS stain (due to the presence of mucin).



3. Undifferentiated Small Cell Carcinoma

WHO classification recognizes 3 histological subtypes:

- **Oat cell carcinoma.**
- **Small cell carcinoma** – intermediate type.
- **Combined Oat cell carcinoma** (with foci of squamous cell and/or adenocarcinoma).

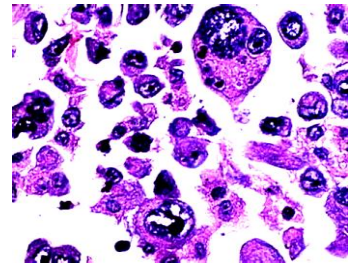


The cellular morphology of small cell carcinoma varies with the method of specimen collection. In sputum, the exfoliated cells are often small in association with long strands of mucous. Dark lines of elongated compact masses of numerous small

hyperchromatic neoplastic cells are seen with satellite single cells scattered around. The nucleus could be degenerated and pyknotic. In brushings and FNA samples, cells are usually better preserved, appearing larger and displaying more variations in nuclear chromasia and chromatin patterns.

4. Undifferentiated Large Cell Carcinoma

These are epithelial tumours comprised of malignant cells with large nuclei and prominent nucleoli that show no evidence of squamous, glandular or small cell differentiation.



In the WHO classification, two variants are recognized:

- **Giant cell carcinoma** which contains a prominent component of highly pleomorphic multinucleated cells which maybe actively phagocytic. The hyperchromatic nuclei show irregular chromatin clumping and large prominent macronucleoli.
- **Clear cell carcinoma** which is a rare variant composed of large cells with clear or foamy cytoplasm without mucin.

In general, during the evaluation of a smear, the cytopathologist should look for the following changes:

- Modification of the normal cells (nuclear and cytoplasmic changes).
- Cell Maturation status.
- Quantity of the cellular material (hypo- or hyper-cellular)
- Mode of desquamation (clusters, sheets or individually scattered cells).
- Background of the smears (i.e., the presence of leukocytes, histiocytes, RBCs, necrotic cells and/or protein deposits).