
Sir William Osler (1849-1919)

The Osler Institute

Excellence in Continuing Medical Education

Cytology Review Course Notes

Disc 1

Disc 2

Disc 3

Disc 4

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Cytology Review Course Disc 1 Notes

Basic Cytology Principles

Respiratory Cytology

Endocervical Cytology

GYN Cytology I

GYN Cytology II

General Cytology

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Basic Cytology Principles

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I. Basic Cytology Principles

A. Introduction

1. Cytology is the study of cells, their structure and function.
2. Four elements, the cell **nucleus**, **cytoplasm**, **membrane** and **extracellular matrix** comprise the basis of the study of diagnostic cytology.
3. A general principle of cytodiagnosis is that the **nucleus** reflects the state of health of a cell (normal, inflamed, or degenerated, as well as hyperplastic, metaplastic, dysplastic, or neoplastic) while the **cytoplasm** reflects the origin and functional differentiation of the cell (mucus-producing glandular cell, squamous cell). When properly evaluated, the nucleus and cytoplasm reveal a wealth of information about the cell.

B. Cell size

1. Cells are often referred to as small, medium, or large.
2. The size is compared to a normal cell or its nucleus (RBC, PMN, lymphocyte, intermediate squamous cell)
3. A small cell is 2-2.5x, medium cell is 3-6x, a large cell is 6-10x , and a giant cell > 10x the size of a lymphocyte.

C. Cell shape, type and composition

1. Abnormal shapes are common in neoplasia (tadpole, hobnail)
2. Monophasic, biphasic and triphasic relate to numbers of cell lines presenting different morphologic patterns in a given neoplasm.
   a. **Triphasic** WT (epithelial, stromal, blastemal)
   b. Biphasic FA, PA
   c. SS is referred to as **monophasic** if only one pattern is present, rather than the usual biphasic appearance with epithelial and d fibroblast-like spindle cells
3. Neoplasms can be of **mixed** cell types (adenosquamous)

D. Nucleus

1. A common feature of cancer cells is “thickening of the nuclear membrane” due to increased chromatin, hyperchromasia with margination and condensation of chromatin beneath the inner aspect of the nuclear envelope.

2. **Chromatin** is present as extremely slender threads (**euchromatin**) with periodic dense knots (**heterochromatin**). **Heterochromatin** is functionally **inactive**.
   a. Heterochromatin stains basophilic with the Pap stain, whereas euchromatin stains faintly.
   b. Normal, non-mitotic cells have a fixed amount of DNA
   c. **Hyperchromatic nuclei** have more (inactive) heterochromatin, while **hypochromatic** nuclei have more (active) euchromatin.
   d. In cancer cells, however, nuclei are often hyperchromatic due to an actual increase in the amount of DNA, being **polyploid** or **aneuploid**
   e. The **size of the nucleus** correlates with the **functional activity** of the cell: in general, the larger the nucleus, the more active the cell.
   f. Chromatin clumping is an important feature. The chromatin clumps may be either fine or coarse, and regularly or irregularly distributed in the nucleus.
   g. Chromatin may dissolve – **karyolysis**, as in the anucleate squame, or form a compact mass - **pyknosis**, as in a superficial cell.
   h. In viral infections the chromatin may become homogenized – **ground glass** appearance.
   i. In early cellular degeneration the chromatin forms multiple, more regular, rounded particles, which may sometimes migrate to the center or the periphery of the nucleus.
   j. **Cancer** is also associated with **chromatin clumping**, however the clumps are irregular in size, shape and distribution.
   k. In **degeneration**, the chromatin often appears **smudgy**, while in cancer the chromatin appears crisp and distinct.
   l. Irregular chromatin distribution in the nuclear "quadrants" is suggestive of malignancy.
   m. In cancer there is also variation not only in one nucleus, but also from nucleus to nucleus.
   n. Marked variation of any cellular feature among cells is a hallmark of malignancy.
   o. In **malignancy**, the **nucleus stains blue**; in **inflammation** it **stains red**!
   p. Nuclear vacuolization is common after radiation.
   q. An early response to irritation (inflammation, thermal, radiation), the nucleus enlarges; however there is little or no hyperchromasia unless induced by radiation. In addition, the cytoplasm also shows changes, such as vacuolization.

r. Nuclear changes diagnostic of cell death are **Pyknosis** (complete clumping of the chromatin into a single dot-like mass), **Karyorrhexis** (pyknotic material breaks up into particles with dissolution of the nuclear membrane), and **karyolysis** or chromatolysis (in which the nuclear material dissolves). In karyolysis, a pale empty space may be seen where the nucleus used to be: the nuclear ghost.

3. **Abnormal mitotic figures** correlate with **aneuploidy**.
   - Aneuploidy is a good indicator in favor of neoplasia, both malignant and premalignant (precursors).

E. Nucleoli

1. They vary in size, number, and shape, but are usually small (micronucleoli), few, round and oval.
   a. **Nucleoli** are mostly protein, and **stain red**; chromocenters stain blue.
   b. Nucleoli are smooth and round; chromocenters irregular and angular.
   c. Nucleoli are often enlarged, multiple and irregular in cancer.
   d. **Macronucleoli** (as large as, or larger than a red blood cell) are usually indicative of malignancy.
   e. Diagnostic rule of thumb:
      1) Conspicuous nucleoli are seen at 40x/high power
      2) Prominent nucleoli are seen at 10x/scanning power
      3) Macronucleoli should be about the size of RBCs
   f. **More than 6 nucleoli** per nucleus, and **irregular** shapes (stars, bananas) suggest a diagnosis of cancer.
2. **Irregular nuclear membranes** are an important diagnostic feature of malignancy.
   - Folded, convoluted nuclear membranes of malignancy must be distinguished from crenation/degenerative changes. The former has relatively smooth, convex hills and valleys, either like the brain or like cookie cutter bites; the latter have wrinkling, irregular, concave spikes, like a star.
3. Increased mitotic activity, particularly **atypical mitotic figures** (tripolar, tetrapolar) are associated with malignancy.
   a. In cancer, there is loss of coordination between the nucleus and the cytoplasm. The nucleus may divide before the cytoplasm is ready, leading to increased **nuclear/cytoplasmic ratios**.

b. In cancer, the nucleolus remains active, leading to small cells with prominent or **macronucleoli**.
4. **Aneuploidy** is often associated with malignancy, leading to variation in nuclear size (anisonucleosis) and staining.

F. Intranuclear pseudoinclusions (INCIs)

1. In intranuclear cytoplasmic invaginations, a portion of the cytoplasm pushes into the nucleus, but lies outside the nuclear membrane (not free in the nucleoplasm) Ultrastructurally cytoplasmic organelles can often be demonstrated in these inclusions.
2. INCIs are seen in a large number of normal as well as hyperplastic and neoplastic cells.
3. Sometimes they are diagnostically important, as in PTC and MM.

G. True intranuclear inclusions

- Viral Cowdry type A and B inclusions

H. Multinucleation

1. Multinucleation and multinucleated giant cells can be seen in response to inflammation and radiation.
2. Multinucleation can result from either endomitosis or cell fusion.
3. **NEVER** diagnose cancer based only on features seen in naked nuclei.

I. Cytoplasm

1. Characteristics of cytoplasm, such as amount, texture, secretory products, and staining qualities are all significant.
   a. Many neoplasms are typed or characterized by **cytoplasmic qualities** (clear cell, oncocytic/granular, signet ring cell type, mucinous, keratinizing), or **shape** (round cell, spindle cell, and pleomorphic cell tumors)
   b. Some metaplastic and neoplastic cells become filled with numerous mitochondria, have abundant finely granular cytoplasm and are known as oncocytes, their tumors are called **oncocytomas**. (The mitochondria are biochemically defective.)
   c. **Mitochondria** can be stained with iron hematoxylin, phosphotungstic acid hematoxylin (PTAH), or acid fuchsin.
   d. Giant mitochondria can be seen in hepatocytes in alcoholic hepatitis or cirrhosis, HCC; skeletal muscle myopathies and some nutritional deficiencies.
2. **Different cell types** have different functional activities and aid in identification:
   a. Glandular cells secrete mucin, and possess secretory vacuoles

b. Mature squamous cells form keratin and have dense hyaline cytoplasm
c. Liver cells produce bile
d. Melanocytes form melanin pigment

3. Specific **identification of cell products** (and other antigens) by immunocytochemistry is of growing importance. Cell products are also identified by electron microscopy.
   a. Dense core granules in neuroendocrine tumors
   b. Melanosomes and premelanosomes in melanoma
   c. Zymogen granules in metastatic pancreatic (acinar) carcinoma
4. **Lipofuscin** accumulates with age, and is known as the "wear and tear" pigment. It is PAS and fat stain positive.
   - The presence of lipofuscin in a cell implies that the cell is slow growing and benign (brown lipofuscin pigment in hepatocytes).
5. **Granular cell tumor** is packed with lysosomes.
   - In lysosomal storage diseases, the affected lysosomes vary greatly in size and are filled with indigestible substrate.
6. **Pigment** can be helpful in the diagnosis of tumors. Many, however, are non-specific.
7. **Hemosiderin** is a golden brown, refractile crystalline pigment, and is a storage form of iron.
8. **Hematoidin**, also is derived from hemoglobin, but does not contain iron.
   - Hematoidin is closely related to **bilirubin**, they are both bright yellow to golden brown.
9. **Lipofuscin** is golden brown.
10. **Anthracotic pigment**, derived from carbon compounds, consists of granular, black particles. It is frequently present in alveolar macrophages, "dust cells" Smokers and urban dwellers have an increase in this pigment.
    - **Melanin** pigment is fine, non-refractile, and stains with the Fontana Masson stain.
11. **Reinke crystals** are seen in Leydig cell tumors.
12. **Microfilaments** are 5 to 7 nm in diameter (actin and myosin).
    - **Actin** is abundant in muscle and **myoepithelial cells**. Myoepithelial cells occur around gland acini and ducts. They are important in aspiration biopsy because their presence suggests that the glandular cells were completely contained in their space, and are, therefore, probably benign.
13. **Intermediate filaments** average about 8 to 10 nm in diameter. Many are diagnostically important, including neurofilaments, glial filaments, and tonofilaments.

a. Coexpression of multiple filaments is rare normally, but can be seen in some neoplasms (PA, RCC and mesothelioma).
b. Intracytoplasmic filaments are diagnostically important in some tumors, particularly SCC, where the amount of keratin directly correlates with the degree of differentiation.

14. **Cell shape** depends on various factors. Most cells tend to **round** up **in fluids**.

J. Cell membrane

1. **Microvilli** are associated with glandular lumens; **cilia** are almost always associated with benign cells.
   - **Microvilli** are long and prominent on mesothelial cells (and mesothelioma), squamous cells (and squamous cell cancers – the better the differentiation, the longer the microvilli), melanomas, large cell lymphomas and hairy cell leukemia.
2. **Cilia** are 2 nm in diameter and can be barely seen by light microscopy, in contrast with microvilli, which cannot.
   - The presence of numerous **cilia** virtually guarantees that the cell is **benign** until proven otherwise. Only very rare exceptions occur (certain well-differentiated carcinomas of the ovary, endometrium, pancreas, and stomach).
3. **Intercellular junctions** are completely absent in cells of hematopoetic and lymphoreticular origin, and are usually poorly formed in cells of mesenchymal origin and their neoplasms.
   a. Intercellular junctions play a very important role in cytologic diagnosis.
   b. Epithelial cells and their tumors usually form at least some groups, even though decreased intercellular cohesion is a characteristic of malignancy.
   c. In contrast, lymphomas and leukemias form no groups at all.
   d. Sarcomas and melanomas usually form few or no groups of cells; any groups present are usually only loosely cohesive, and tend to occur around blood vessels.

K. Extracellular matrix

- Groups of epithelial cells are characteristically surrounded by basal lamina as they form structures such as glands. In contrast, mesenchymal cells tend to lie free in the extracellular matrix.
- This can be important in distinguishing a carcinoma, in which groups of cells are invested with **reticulin**

fibers, whereas individual sarcoma cells are invested with reticulin.

1. **Extracellular material** may be present on the cytology slide as the background.
2. The **background** may reflect a normal, inflammatory, dysplastic, or neoplastic process.
3. In **inflammation**, numerous inflammatory cells are present.
4. In trichomonas infection, there is a basophilic, finely granular background.
5. A **tumor diathesis** consists of fresh and old blood, fibrin, and necrotic cellular debris. (Cervical stenosis and pyometra may also produce similar exudates.)
6. A **watery background** (or transudate), is seen in Pap smears in carcinoma of the endometrium or fallopian tube.
7. **Metastatic carcinomas typically lack a diathesis.**
8. **Stroma** may be present, and can be myxoid, fibrillar, chondroid, colloid, contain amyloid…

L. Architectural patterns

1. Papillary, rosettes, follicular/acinar
2. Solid, cystic

M. Ancillary tests

1. These are used to aid the final diagnosis:
2. Histochemical stains
3. Immunohistochemical stains
4. Electron microscopy
5. Flow cytometry, image analysis (ploidy)
6. Molecular studies

N. Summary of diagnostic features of malignancy

1. **Cells**
   a. Usually numerous
   b. Crowded, disorganized groups
   c. Single, intact atypical cells
   d. Cannibalism (cell in cell pattern)
   e. Pleomorphism
   f. Abnormal shapes
   g. Increased nuclear/cytoplasmic ratio
2. **Nucleus**
   a. Enlarged
   b. Loss of polarity
   c. Pleomorphism
   d. Multinucleation
   e. Bare nuclei
   f. Molding
   g. Thick, irregular nuclear membranes
   h. Hyperchromasia
   i. Irregular chromatin

   j. Prominent, multiple, irregular, or macro-nucleoli
   k. Mitotic figures (esp abnormal)
3. **Cytoplasm**
   a. Pleomorphism
   b. Loss of cell boundaries
   c. Abnormal cellular products (keratin, mucin)
4. **Background**
   a. Necrosis
   b. Blood
   c. Tumor diathesis
5. No one criterion is pathognomonic of cancer, but rather a combination of the criteria must be used to make a malignant diagnosis. Selections from nuclear, cytoplasmic and cellular features must be made and added together to make a diagnosis
6. **Benign lesions** usually are:
   a. Sparsely cellular
   b. Cohesive
   c. Regular chromatin
   d. Lack pleomorphism
   e. Ciliated cells are almost always benign

O. Diagnostic pitfalls and limitations

1. Specimen **adequacy**
   a. Examine at low power to determine the quality of fixation and staining, cellularity, architectural pattern, background, and stromal characteristics. Individual cell morphology is studied at higher magnification.
   b. CSF specimens normally contain very few cells
   c. If only fat or blood are aspirated after repeated liver sampling, consider the possibility of fatty change or a cavernous hemangioma, before calling the specimen "unsatisfactory"
2. Certain benign conditions have worrisome cytologic features, for example nodular fasciitis or granulomatous lymphadenopathy.
3. Some benign tumors, such as adenomas of the thyroid or parathyroid may contain highly pleomorphic cells.
4. Some malignant tumors, such as RCC appear deceptively bland.
5. Some neoplasms present diverse morphologic patterns and are great mimics (MM, MTC)
6. Large quantities of fluid may be aspirated from malignant lesions, such as PTC and necrotic SCC. Do not misdiagnose them as branchial cleft cysts.
7. It is not possible to assess the presence or absence of invasion or distinguish in situ from infiltrative carcinoma.

8. Identification of malignant cells is a definitive diagnosis, but a negative report cannot exclude malignancy if there is a question of adequate sampling.
9. It is not possible to diagnose lesions which require histologic criteria, such as follicular adenoma and follicular carcinoma of the thyroid, parathyroid hyperplasia and adenoma, adrenocortical hyperplasia and well-differentiated carcinoma, papilloma vs papillary breast carcinoma.
10. Low grade lymphomas vs lymphoid hyperplasia, primary vs secondary adenocarcinoma in the lung, primary vs secondary hepatic neoplasms are a problem. Flow cytometry and immunohistochemistry may be helpful.
11. Failure to differentiate between screening and diagnostic cytologic sampling.
12. Floaters or contaminants from accompanying cytologic material (cross-contamination) is a problem, particularly with body fluid specimens and must be minimized. Often it is difficult to be completely sure that the problem is really contamination rather than actual abnormality. Further evaluation of the patient with repeat specimens may be needed.
13. Whenever possible neoplasms should be accurately typed, as treatment modalities vary for different neoplasms.
14. Ancillary tests should be performed when applicable and with discretion. Most ancillary tests are performed to type a neoplasm, which must first be diagnosed as benign or malignant on a routinely stained preparation. Sometimes, despite multiple testing, precise identification of a primary source in cases of disseminated tumor or an occult primary tumor may not be possible.

P. Common causes of error

1. Insufficient clinical information. Whenever possible, the clinical and radiologic impressions must be correlated with the cytologic findings – “triple test”
2. Failure to realize the specimen is not representative of the lesion (the target was missed)
3. Trying to make a diagnosis from unsatisfactory specimens
4. Excessive inflammation or necrosis
5. Failure to correlate with previous cytologic/histologic specimens
6. Failure to recognize a diagnostic pitfall

Q. Stains

- Traditionally, the Papanicolaou stain is used for exfoliative cytology. The nuclear details are crisp, and it is also suitable for aspiration cytology specimens.

- Other stains used for FNA specimens are Romanowsky stains (Diff-Quick, Giemsa) and Hematoxylin and Eosin.

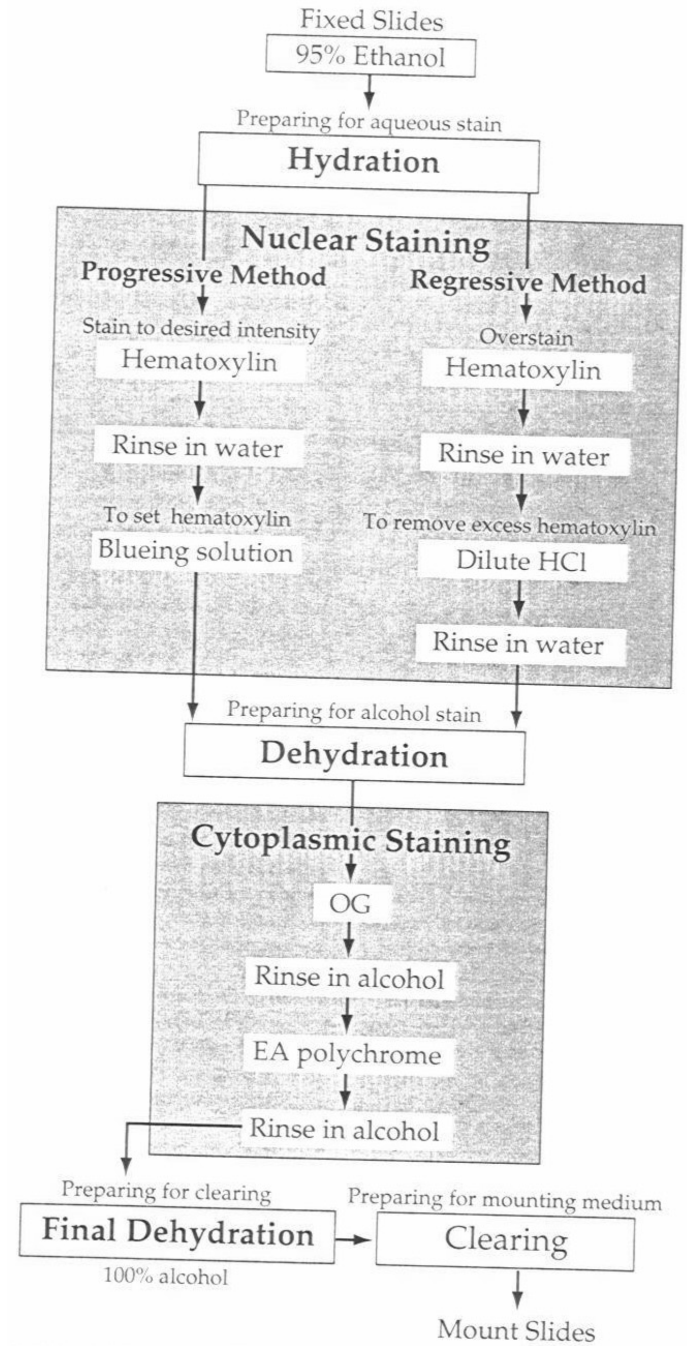
1. The ideal stain should allow for:
   a. architectural pattern evaluation
   b. nuclear evaluation
   c. cytoplasmic details
   d. identification of background features (stroma, secretions)

R. Papanicolaou staining method

- This shows the variations in cell morphology according to the degree of cell maturity and cell activity.

1. The main advantages are:
   a. Nuclear detail
   b. Cytoplasmic transparency
   c. Cell differentiation
2. The main steps are:
   a. Fixation
   b. Hydration, dehydration
   c. Nuclear staining with hematoxylin
   d. Cytoplasmic staining with counterstains orange G and EA
   e. Rinsing, clearing and mounting
3. **Fixation**
   a. 95% ETOH or equivalent
   b. Wet fixation (slide immersion)
   c. Coating or spray fixatives (alcohol and carbowax mixture)
4. **Stains**
   a. Nuclear stain – hematoxylin
   b. Cytoplasmic counterstains – Orange G and EA
5. **Hematoxylin**
   a. Nuclear stain
   b. A natural dye
   c. Water based
6. **EA stain**
   a. Polychrome stain composed of
      1) Bismarck brown Y
      2) Light green SF yellowish
      3) Eosin Y
      4) Phosphotungstic acid and lithium carbonate
   b. Alcohol based
   c. Stains cytoplasm, nucleoli, cilia
   d. EA 36, EA 50, EA 65, etc.
7. **Orange G stain**
   a. G (Gelb) German for yellow
   b. OG-6 (6^{th} variant)
   c. Stains keratin

8. **Progressive vs regressive methods**
   a. Applies primarily to hematoxylin component
   b. Same outcome if done correctly
   c. Progressive: stained until required optical density is achieved, does not stain background
   d. Regressive: overstains entire cell. Requires acid bath to extract excess. Greater variability of results, less "forgiving", less suitable for non-GYN samples, which do not adhere as well to the slides.
9. **Hydration/dehydration**
   a. Hydrations needed for transition between alcohol fixation and water based hematoxylin stain
   b. Dehydration needed for transition between water based hematoxylin and alcohol based cytoplasmic stains
10. **Bluing**
   a. Required for both progressive and regressive staining
   b. An alkaline bath compared to more acidic hematoxylin
   c. Scott's tap water, ammonium hydroxide, lithium carbonate
11. **Rinsing/clearing**
   a. Rinsing with water after the hematoxylin stain
   b. Rinsing with alcohol after cytoplasmic stains
   c. Complete clearing with 100% alcohol
   d. Final bath in xylene (or xylene substitute) for transparency
12. **Coverslipping**
   a. Glass coverslips – number 1 thickness, 24x60
   b. Cautions: air bubbles, correct side, cross contamination
13. **Quality assurance**
   a. Regular filtering, changing of stains and reagents (recommended that dyes be changes after 2000 slides or 6 to 8 weeks, whichever comes first)
   b. Daily check of stain quality
   c. Separate staining of GYN and non-GYN samples
   d. Solutions should be kept covered when not in use
   e. Documentation!
14. **CSF**
   - Separate equipment and staining setup is recommended to prevent contamination from other fluid samples, which are more cellular.


Fixed Slides
95% Ethanol
Preparing for aqueous stain
Hydration
Nuclear Staining
Progressive Method
Regressive Method
Stain to desired intensity
Hematoxylin
Rinse in water
To set hematoxylin
Blueing solution
Overstain
Hematoxylin
Rinse in water
To remove excess hematoxylin
Dilute HCl
Rinse in water
Preparing for alcohol stain
Dehydration
Cytoplasmic Staining
OG
Rinse in alcohol
EA polychrome
Rinse in alcohol
Preparing for clearing
Final Dehydration
100% alcohol
Preparing for mounting medium
Clearing
Mount Slides

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Respiratory Cytology

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I. Exfoliative Respiratory Cytology

A. Diagnostic triad

1. **Sputum**
   - Early morning specimens (3 for diagnosis, 5 to exclude carcinoma)
   - Increased sensitivity with central lesions
   - Cannot localize lesions
   - Best for the diagnosis of squamous cell carcinoma and small-cell carcinoma, less accuracy for metastatic disease, lymphoma and adenocarcinoma
   - Often difficult, hampered by cellular degeneration
   - Sensitivity for detecting malignancy: 40 to 60%

Specimen preparation:
Saccomanno Blender vs "Pick and Smear" Techniques

	Saccomanno	"Pick and Smear"
Method	Cells are collected in 50% ethanol and 2% polyethylene glycol (Carbowax), specimen is broken up using a blender, centrifuged and smears are prepared. Note: a direct smear should also be performed.	Fresh specimen is preferred. Strands of dense or bloody material are selected and smeared onto a slide.
Sensitivity/ Specificity	No difference between methods	No difference between methods
Number of Cells Sampled	Concentrates cells and can provide a higher diagnostic yield	Fewer cells sampled per prepared slide.
Small-cell CA	Blender disperses cells making it more difficult to detect.	Easier to detect
Fungal Hyphae	Dispersed and broken	Intact
Fixation	Better	For nonfresh samples, ethanol fixation may obscure cytologic detail.

2. **Bronchial cytology** – bronchial brushings and washings
   - Better for central lesions
   - Can localize lesions
   - Smaller tumors can be diagnosed.
   - Sensitivity for detecting malignancy: 70to 85%
3. **Postbronchoscopy sputum**
   - May have the highest diagnostic rate of any respiratory cytology specimen
   - Often contains degenerated/atypical bronchial cells mistaken for carcinoma

B. Utility as a screening test (controversial)

1. Accurate (specificity (tumor typing) and sensitivity (tumor finding))
2. Not cost effective even in high-risk patients
3. No increase in survival
4. Role
   - Detection, conformation and typing (primary and metastatic)
   - Posttherapeutic monitoring
   - Complementary procedure to radiologic exams
   - Benign disease and opportunistic infections

C. Factors affecting diagnosis (false negative)

1. **Number of specimens**
   - 3 satisfactory sputum specimens detect approximately 60% of cancer.
   - 3 bronchial specimens will detect up to 90%.
   - 1/2 to 3/4 of tumors are diagnosed by the first specimen.
   - 15% to 20% of tumors on the second
   - 10% on the third
   - **Optimum number of specimens is three to five.**
2. **Location of tumor**
   - Central tumors most easily detected
   - Bronchial cytology is better than sputum for peripheral lesions.
3. **Size of the tumor**
   - Optimal tumor size for diagnosis 3 to 6 cm
4. **Type of tumor and degree of differentiation**
   - Squamous cell and small-cell carcinomas more accurately classified than adenocarcinoma and bronchioloalveolar carcinoma
   - Adenocarcinoma is the least commonly detected and least accurately classified.
   - Bronchial cytology can distinguish small-cell from nonsmall-cell carcinoma 100% of the time.
   - Sputum better for squamous and bronchial better for adenocarcinoma

D. False positives (rare)

1. Treatment can be initiated on the basis of a cytologic diagnosis without confirmatory biopsy.
2. Causes of FP
   - Atypical squamous metaplasia
   - Repair/regeneration
   - Pneumonia, especially necrotizing pneumonia
   - Infarcts
   - Chronic irritation (asthma, tracheostomy stoma, chemo)

E. Adequacy

1. Sputum
   - Alveolar macrophages
   - Bronchial cells are insufficient determinants of accuracy.
2. Bronchial washings/brushings
   - Numerous bronchial cells
3. Bronchioloalveolar lavage
   - Abundant alveolar macrophages
   - Squamous cells should be few.

F. Other specimen types

1. **Bronchoalveolar lavage**
   a. Good for detecting peripheral lesions
   b. Sensitivity for detecting malignancy: 70to 85%
   c. Most useful for the diagnosis of
      - Opportunistic infections
      - Interstitial lung disease
      - Inflammatory processes
      - Lymphocytes predominate (sarcoid, hypersensitivity pneumonitis and berylliosis)
      - Neutrophils/macrophages predominate (idiopathic pulmonary fibrosis, cytotoxic drug reactions, Langerhan's histiocytosis)
      - Granulomatous diseases
      - Pulmonary hemorrhage – hemosiderin laden macrophages, however also seen in infections
      - Carcinoma
2. **Imprint cytology**
   - Performed at the time of flexible fiberoptic bronchoscopy
   - Increases the diagnostic sensitivity (approaches 100%) of the procedure when used in conjunction with brush and biopsy
3. **Pulmonary microvascular cytology**
   - A blood sample is drawn through a wedged pulmonary artery to diagnosis lymphatic carcinomatosis.
   - Specimen is placed on a Ficoll-Hypaque gradient to remove blood and concentrate the cells.
   - Megakaryocytes indicate a satisfactory specimen.
4. **Cell blocks**
   - Inferior cytologic detail
   - Usually not recommended for routine specimens, good when a battery of special stains is needed.

II. Normal Constituents

A. Squamous cells

1. More common in sputum specimens
2. Represent oral contamination
3. May demonstrate mild cytologic atypia

B. Goblet cells

1. Vacuolated cytoplasm
2. Usually there are 5-10 ciliated cells for every goblet cell.
3. Naked nuclei are common.

C. Ciliated columnar cells

1. Rare in sputum samples
2. Common in brushing and washing samples
3. Nucleus is polarized and has a finely granular chromatin, may occasionally have a small nucleolus.

D. Reserve cells

1. Undifferentiated epithelial cells (past board question)
2. Round lymphocyte-like cells with a central, hyperchromatic nucleus
3. Scarce in sputum, may be seen in reserve cell hyperplasia
4. In brushings may be associated with bronchitis or aggressive abrasion of the mucosa with a brush.

III. Benign Reactive/Reparative Changes

A. Reactive atypia

1. Enlarged, pleomorphic nuclei with coarse chromatin and prominent nucleoli
2. Cellular cohesion with fewer single cells
3. Often associated with inflammation
4. CILIA
    - A ciliated cell is benign (except for metastatic ovarian carcinoma).
    - Terminal bar is usually a sign of benignity (except for colorectal carcinoma, bronchioloalveolar carcinoma).
    - Malignant cells growing underneath the surface epithelium may exfoliate together (especially small-cell carcinoma).
5. Multinucleation
    - Common nonspecific reaction of bronchial cells
6. Regenerative/reparative bronchial cells
    - May mimic cancer with bizarre cytologic atypia
    - Cell enlarges but the nuclear to cytoplasmic ratio remains the same.
    - No crowding or architectural disarray
7. Ciliocytophthoria
    - Broken off tops of bronchial cells
    - Nonspecific, often associated with viral infections
8. Pneumocytes
    a. Type I
        - Flat lining cells of alveolus
    b. Type II
        - Seen in reparative conditions
        - Not usual in respiratory specimens unless hyperplasia
        - Pulmonary infarcts, viral infections, pneumonia

- Singly or in clusters
- Vacuolated cytoplasm
- Increased nuclear to cytoplasmic ratio
- Often confused with adenocarcinoma

IV. Noncellular Material

A. Curschmann's spirals

- Associated with excess mucous production
- Asthma and smoking
- Deep lung specimens
- Dark staining center with lighter periphery

B. Ferruginous bodies

- Form as iron slats precipitate onto inhaled dust
- Usually asbestos, but may form around fiberglass, carbon

C. Alveolar proteinosis

- PAS positive, mucicarmine negative
- Dirty background with coarsely granular, eosinophilic debris

D. Elastin fibers

- Tissue destruction, abscess, infarct, carcinoma
- Elongated, frayed, poorly stained often refractile

E. Amyloid

F. Psammoma bodies

G. Vegetable material

H. Charcot-Leyden crystals

- Needle-like red crystals composed of condense granules from eosinophils
- Seen in allergy, allergic bronchopulmonary aspergillosis

I. Contaminants

- Talc
- Pollen
- Alternaria

J. Pneumoconiosis

- Hemosiderosis – marked increase in hemosiderin laden macrophages
- Loffler's pneumonia – eosinophils
- Silicosis – weakly birefringent, silvery particles
- Silicate pneumoconiosis – brightly birefringent silicates
- Asbestosis – ferruginous bodies suggestive
- Berylliosis – cannot be seen
- Anthracosis – carbon
- Talc – strong birefringence, no Maltese cross
- Starch – Maltese cross (glove powder)

V. Benign Proliferations

A. Reserve cell hyperplasia

- Response to chronic irritation
- Bronchial brushings

- Uniform small dark nuclei with a rim a basophilic cytoplasm
- Tightly cohesive groups
- No nucleoli unless irritation
- D/Dx small-cell carcinoma

B. Squamous metaplasia

- 20-80% of all patients
- Atypical squamous metaplasia is associated with necrotizing pneumonias, often resulting in a false positive diagnosis.

C. Creola bodies

- Exfoliated clusters of pseudopapillary, three dimensional groups of reactive/atypical bronchial cells
- Conspicuous in asthmatic patients

VI. Inflammation

A. Acute inflammation

B. Chronic inflammation

C. Granulomatous inflammation

D. Radiation injury

- Cytomegaly
- Multinucleation
- Macronucleolation
- Vacuolization

VII. Infections

A. Bacteria

- Acute inflammation present

B. Viral infections

1. Herpes
2. CMV
3. Adenovirus
4. Measles
5. Parainfluenza
6. Respiratory syncytial virus
7. Characteristics of viral infections
    - Interstitial infiltrates
    - Mononuclear cell infiltrates
    - Edema
    - Pneumocyte hyperplasia/atypia

C. Mycobacteria

D. Fungi

1. Histoplasmosis
    - Yeast form in host.
    - 2-4 microns in diameter
    - Narrow neck buds
    - Often intracellular
2. Coccidiomycosis
    a. Spherules

- 5-30 microns, immature
- 30-100 microns, mature

b. Endospores 2-5 microns

3. Cryptococcosis
   - Yeast form in host, 4-10 microns, variation in size and shape.
   - Mucin positive capsule
   - May be intracellular or extracellular
   - Capable of colonizing
4. Blastomycosis
   - Broad-based budding
   - Yeast form in host, 5-15 microns.
5. Aspergillosis
   - Hyphal form in host.
   - Colonizing infection
   - 2-5 microns in diameter
   - Septate, dichotomous, 45° branching
6. Mucormycosis
   - Hyphal form in host
   - 10-25 microns in diameter
   - Sparsely septate, 90° branching
7. Candidiasis
   - Yeast form 2-6 microns in diameter.
   - Pseudohyphae 2-4 microns in diameter
8. Pneumocystis
   - GMS can stain RBCs (pitfall).

VIII. Malignant Disease

A. Squamous cell carcinoma

1. Arise centrally, can often be diagnosed on sputum
2. Keratinizing (well-differentiated)
   a. More single cells
   b. Pleomorphic, bizarre
   c. Stains orange-pink
   d. Pearls, rings, ghost
   e. Low to high N/C ratio
   f. Hyperchromatic nuclei, less prominent nucleoli
   g. D/dx: inflammatory squamous atypia
      - Pneumonia, fungal infections, infarct, tracheostomy tubes
3. Nonkeratinized (poorly differentiated)
   - More groups
   - Cells are more uniform, less bizarre.
   - Stains blue-green
   - No pearls, occasional dyskeratotic cells
   - Coarse chromatin, more open, more prominent nucleoli

   a. D/dx
      1) Repair/regeneration with pleomorphic nuclei and nucleoli
      2) Pemphigus

3) Small-cell carcinoma
   - Little nuclear molding
   - Nucleoli
   - Little crush artifact

B. Adenocarcinoma

1. Arise peripherally, better diagnosed by bronchial cytology
2. Bronchogenic adenocarcinoma
   - Crowded sheets, cell balls, papillae, microacini
   - Three dimensional groups
   - Polar orientation
   - Lobulated border
   - Vesicular chromatin
   - Prominent nucleoli
   - Foamy, granular, cytoplasm
   - +/- Cytoplasmic mucin
3. Bronchioloalveolar carcinoma
   - Sputum cytology has the highest specificity and sensitivity.
   - Usually abundant cellularity
   - Three dimensional groups with great depth of focus
   - Abundant cytoplasm
   - Finely distributed chromatin, single small nucleolus
4. Large cell undifferentiated carcinoma
   - Large pleomorphic cells
   - Single cells with discohesion
   - No evidence of squamous or adeno differentiation
5. Small-cell carcinoma
   - Small-cells, 1.5–2 times the size of a lymphocyte
   - Conspicuous molding
   - Crush artifact
   - Hyperchromatic to pyknotic nuclei
   - Inconspicuous nucleoli
   - Scant, delicate cytoplasm
   - Tumor diathesis, necrosis
   - Popcorn on a string

   a. D/dx: reserve cell hyperplasia
      - RCH is more cohesive.
      - Little molding
      - +/- prominent nucleoli
      - No diathesis, no crush
6. Other primary tumors

   a. Carcinoid
      - Single cells with discernable cytoplasm
      - Stripped nuclei
      - Plasmacytoid appearance
      - No necrosis, no mitosis, no molding

   b. Granular-cell tumor

   c. Adenoid cystic carcinoma

   d. Mucoepidermoid carcinoma

   e. Oncocytoma
   f. Blastoma
   g. Carcinosarcoma
   h. Sarcoma
   i. Lymphoma
7. Metastasis
   - Metastatic adenocarcinoma is 3 times more common than primary adenocarcinoma
   - Most common mets: GI, breast, lymphoma/leukemia and melanoma

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