CYTOLOGY
Fixation & Staining

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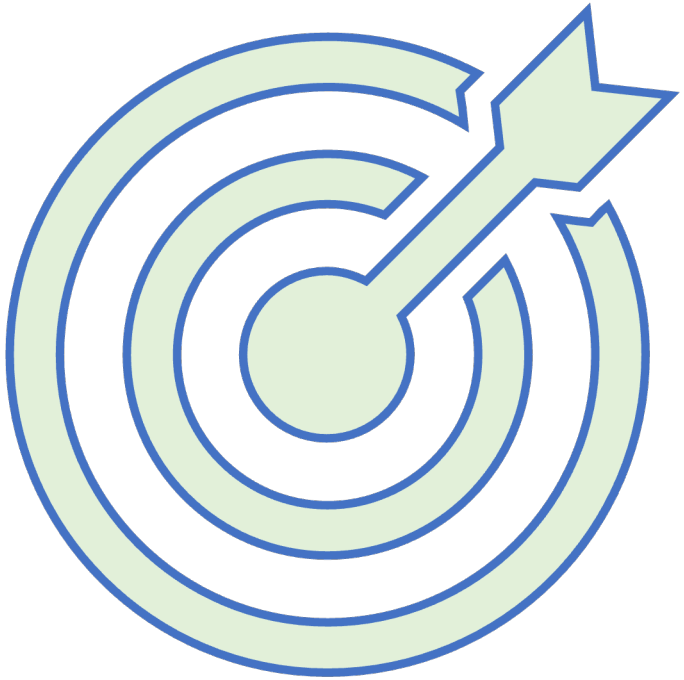
Learning Objectives

1. Define fixation
2. Explain the Aims of Fixation.
3. Describe Fixation Methods & types of fixatives.
4. Describe Cytology Staining for dray fixed slides.
5. Name Other Cytopathology Staining Techniques.
6. Describe Immunocytochemistry.



Definition and Aims of Fixation in Cytology

Definition of Fixation:

Preservation of cells as close as possible to the living state.

The Aims of Fixation

- Preserves cellular architecture and morphology
- **Stope** enzymatic degradation and autolysis
- **Prevents** putrefaction by inactivating bacteria and enzymes
- **Stabilizes** protein structures for better staining
- **Enhances** contrast and visibility of cellular details under microscopy
- **Facilitates** accurate diagnosis by maintaining cell integrity

An appropriate fixative for Cytodiagnostic purposes should perform the following functions

- ✓**Penetrate** cells rapidly
- ✓**Minimize** cell shrinkage
- ✓**Maintain** morphologic integrity
- ✓**Deactivate** autolytic enzymes
- ✓**Replace** cellular water
- ✓**Facilitate** diffusion of dyes across cell boundaries
- ✓**Help** cells adhere to a glass surface
- ✓**Provide** consistent results over time

Fixation Methods

1. Wet Fixation:

Wet fixation in cytology refers to preserving cells by immediately immersing the cytology specimen in a liquid fixative after it has been collected.

2. Air drying:

The process is straightforward; the specimen is spread on a slide and left to dry in the air.

3. Heat Fixation:

It is rarely used in cytology but can be applied for certain types of smears to adhere cells to the slide.

Fixation Methods

Wet Fixation:

1. **Alcohol-based Fixatives** are 95% ethanol or methanol, dehydrate, and coagulate proteins to preserve cell morphology.
2. **Formalin-based Fixatives:** Formaldehyde solution that cross-links proteins, commonly used for tissue specimens but also for specific cytological preparations.
3. **Spray Fixatives:** Aerosols that rapidly fix cells on slides often contain a mixture of alcohol and polyethylene glycol.

Time of Fixation

- ✓Minimum 15 minutes of fixation
- ✓Can be Prolonged
- ✓several days or even a few weeks
- ✓If smears are to be preserved over a long period in alcohol, storing them in capped containers in the refrigerator is better.

The most common fixatives

Coating fixatives

Carbowax Artifact

Carnoy’s fixative

Coating fixatives

- Coating fixatives in cytology are substances used to 'coat' or 'cap' cellular and tissue components, stabilizing them structurally and chemically for microscopic examination.
- Coating fixatives are generally easy to apply, often coming in a spray form that can be evenly distributed over the slide: aerosols or liquid base.
- **Diaphine** fixative Spray coating fixative (Hairspray)
- Not recommended for bloody smears

Dual action:

Alcohol base	-	fixes the cells
Wax like substance	-	forms a thin protective coating

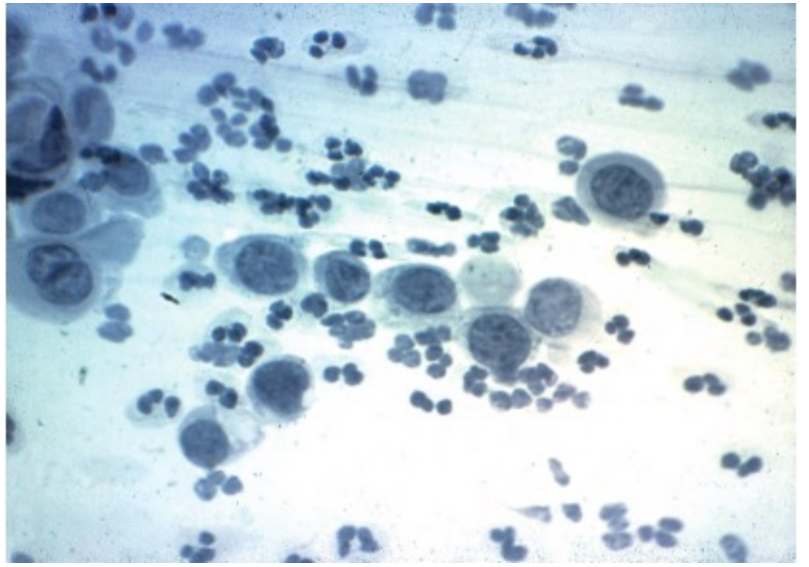
Carbowax Artifact

Carbowax (Polyethylene Glycol) fixative is often used to prepare specimens for **electron microscopy** due to its protective properties that help **preserve ultrastructural details**.

It can help preserve cell shapes and structures without causing significant shrinkage or distortion, which is beneficial for maintaining the morphological details required for accurate cytological assessment.

If the carbowax is not removed Completely:

Nuclei will then appear foggy and lack chromatinic detail, and the cytoplasm may exhibit a pale blue color.



Carbowax not removed. Lack of chromatin details and hazy appearance of the cell

Carnoy's fixative

A mixture of chloroform, Absolute alcohol, and acetic acid preserves fine cellular details. 6 : 3: 1

Carnoy's fixative is designed for use with hemorrhagic samples.

An **excellent nuclear fixative** as well as a preservative of **glycogen**

Fixation Methods

2. Air drying Fixation :

1. **Morphological Changes:**

   Air drying can cause morphological alterations.

2. **Staining Compatibility:**

   Air-dried slides are often stained with **Romanowsky-type stains**, such as Wright's, Giemsa, or Diff-Quik**.**

3. **Use Cases:**

   Air drying is commonly used in **hematological cytology** and sometimes in **fine needle aspirations**, where the particular staining qualities of air-dried cells benefit the diagnostic process.

4. **Rapid Assessment:**

   This method allows for a rapid on-site preliminary assessment of the sample (e.g., adequacy assessment during fine needle aspiration procedures).

Cytology Staining

Dr. Aziza Alrafiah

Characteristics Of Ideal Stain In Cytopathology

1-Enhance contrast: Stains help differentiate cellular components by providing color contrast.

2- Proper Cellular detail: Different stains highlight specific parts of the cell, such as the nucleus, cytoplasm, or certain organelles.

3- Diagnostic clarity: Staining can reveal pathological changes, aiding in the diagnosis of diseases.

4- Evaluation of **background** (blood, secretions, mucin…….)

Stains used in cytopathology

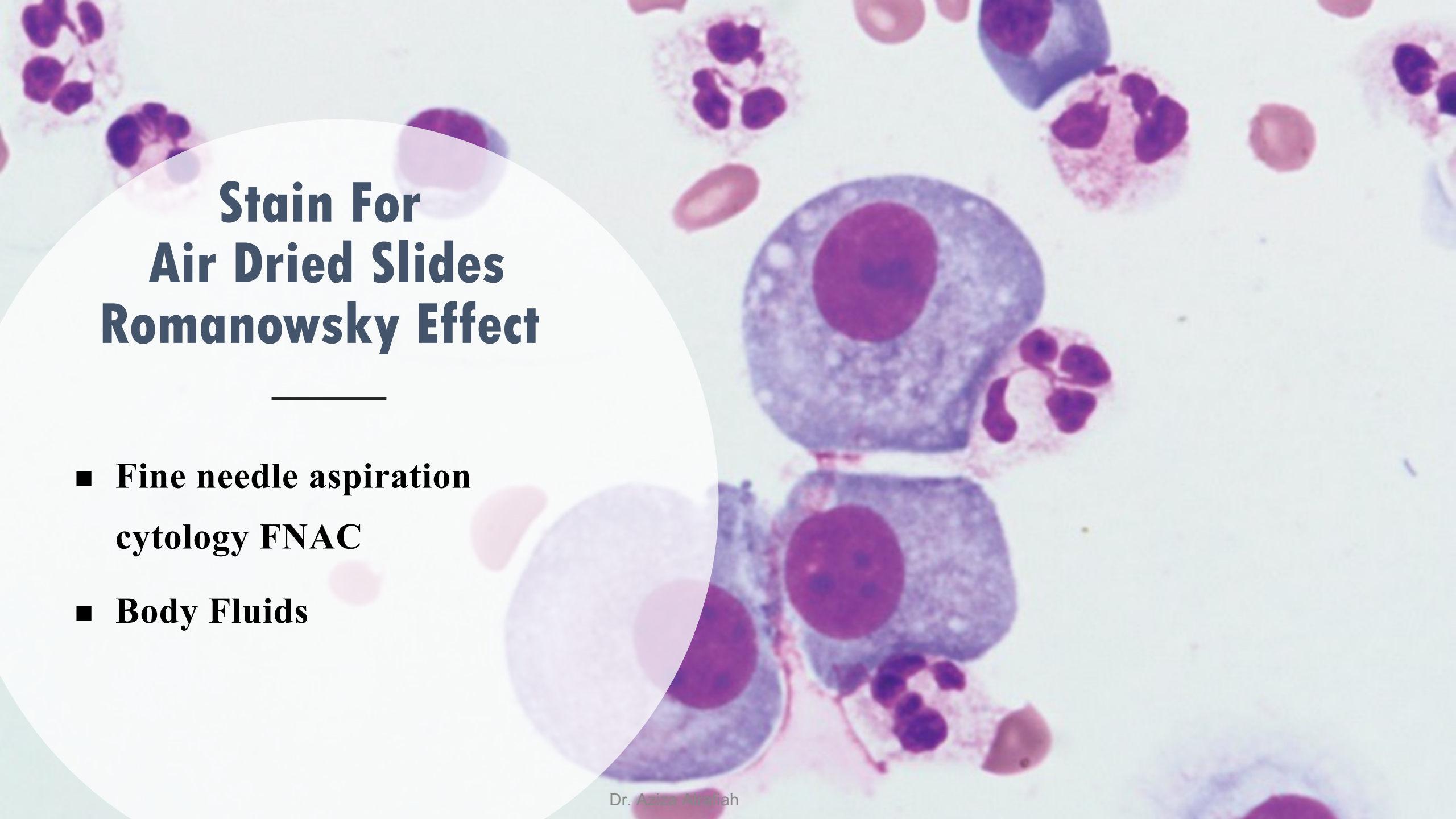
1.Papanicolaou stain

2. Romanowsky stain (FNAC & fluids)

3. H & E (FNAC & fluids)

Stain For Air Dried Slides Romanowsky Effect

- Fine needle aspiration cytology FNAC
- Body Fluids



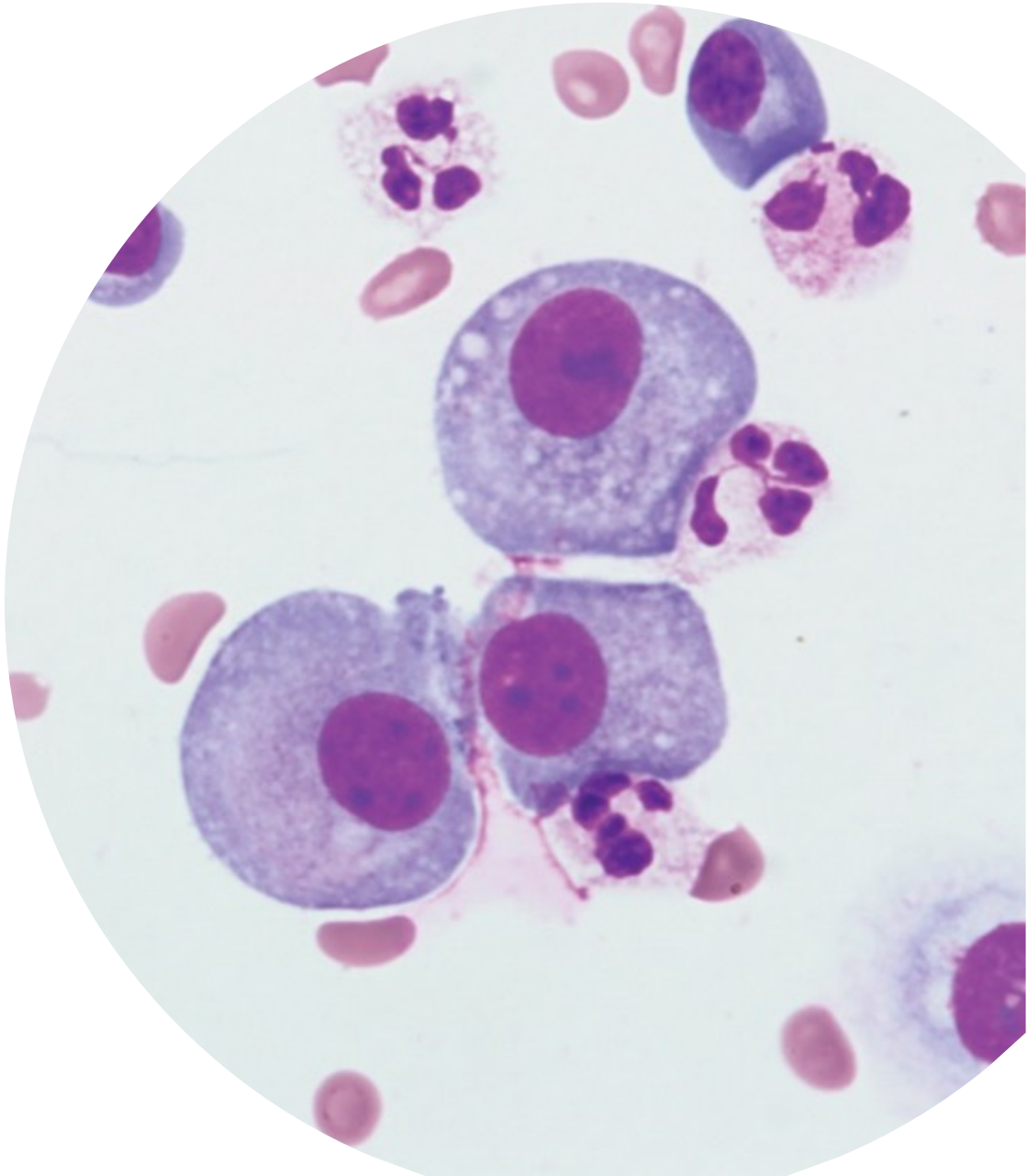
ROMANOWSKY STAIN

Group of stains include:

1. Wright's stain
2. Giemsa stain
3. Wright's Giemsa stain

Most common combination:

1. May Grunwald-Giemsa stain (MGG)
2. Leishman stains
3. Diff- Quik stain



Romanowsky Effect

- **Purple** nuclei
- **Blue** cytoplasm
- **Pink** red blood cells

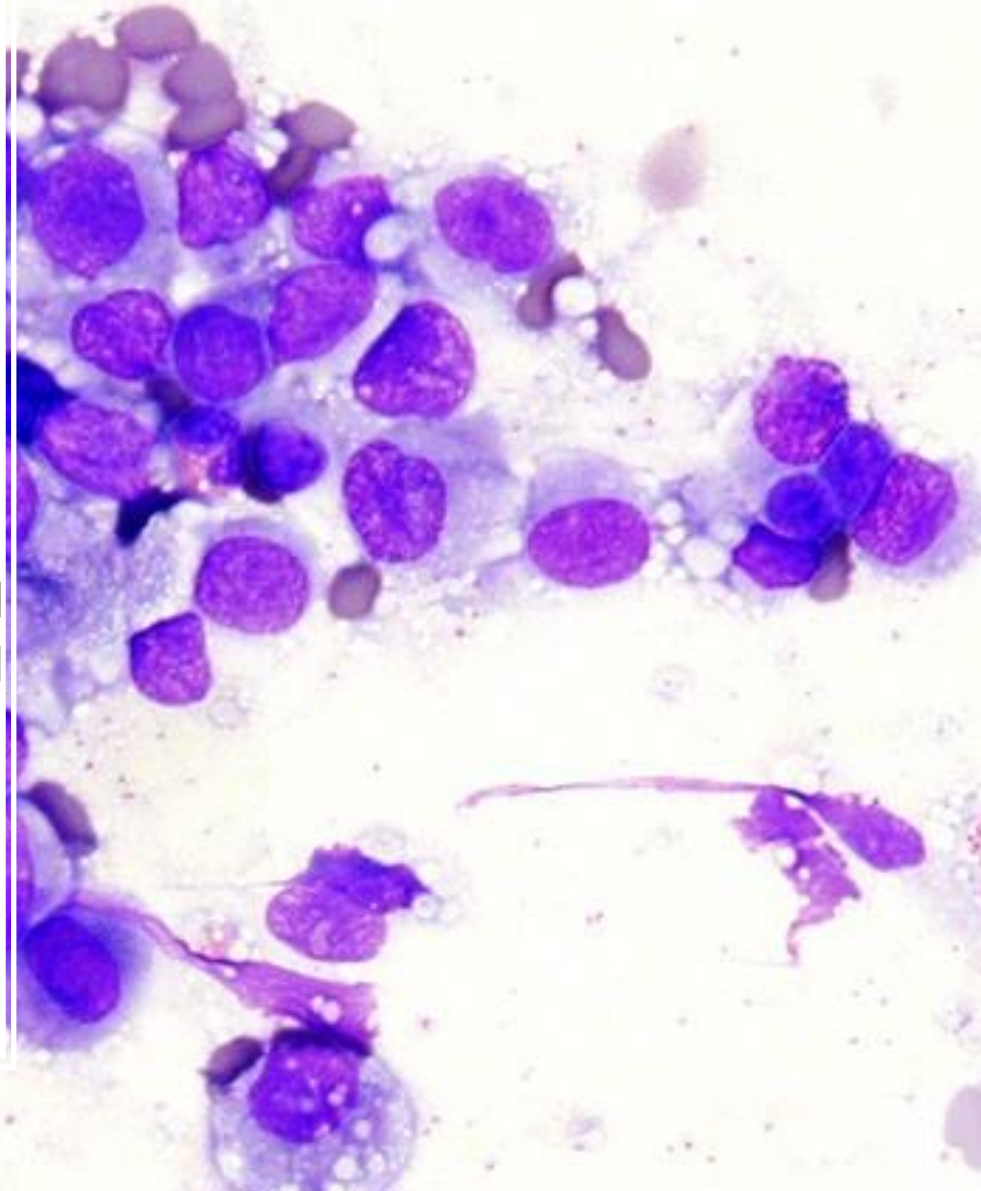
- Requires careful control of PH, Which should be between PH 6.8 to 7.2

- **Staining kits:**
- Rapi-Diff
- Diff-Quik

Diff-Quik Stain

- **On-site stain**
- Allows quick assessment of cell morphology, enabling rapid on-site evaluation of sample adequacy and preliminary diagnosis.
- Is a commercial Romanowsky stain variant.
- commonly used in histological staining to rapidly stain and differentiate a **variety of smears**
- It's used for various sample types, including **blood smears**, **bone marrow aspirates**, and **fine-needle aspiration** biopsies.

Advantages: It provides excellent delineation of nuclear and cytoplasmic elements and is less labor-intensive than traditional stains like Papanicolaou.



Diff-Quik Stain Method

Allow smears to dry

1.Dip slide five times, for one second each, into **Fixative.**

Allow excess to drain after each dip.

2.Dip slide five times, for one second each, into **Stain 1**. Allow excess to drain after each dip.

3. Dip slide five times, for one second each, into **Stain 2**.

Allow excess to drain after each dip.

4. Rinse slide in distilled water or Weise's buffer, pH 7.2.

5. Blot or allow to dry in air.

6. Examine at low power to identify structures and then under oil immersion.

Solutions :
Fixative (Fast green in methanol) - pale green
Stain solution 1 (Eosin G in phosphate buffer) - red
Stain solution 2 (Thiazine dye in phosphate buffer)-blue
Distilled water or Weise's buffer, pH 7.2.

Other Cytopathology Staining Techniques

Besides the widely used Papanicolaou and Diff-Quik stains, cytopathology incorporates various other staining techniques tailored for specific diagnostic purposes.

1. **Acid-fast Stain**: Used to identify acid-fast bacilli such as Mycobacterium tuberculosis in sputum samples.
2. **Giemsa Stain**: Effective for detecting parasites and certain bacteria, as well as for hematologic cytology.
3. **Masson's Trichrome**: Differentiates between muscle fibers, collagen, and fibrin in tissue samples.
4. **Periodic Acid-Schiff (PAS)**: Highlights carbohydrates and carbohydrate-rich structures, helpful in diagnosing fungal infections and glycogen storage diseases.
5. **Congo Red**: Used for the identification of amyloid deposits in tissue.
6. **Silver Staining**: Used for reticulin fibers, nerve fibers, and fungal organisms.

Other Cytopathology Staining Techniques

1-Immunocytochemistry (ICC)

Immunocytochemistry (ICC) is a common laboratory technique that uses antibodies that target specific peptides or protein antigens in the cell via specific epitopes.

2- Flow cytometry

Flow cytometry is a technique used to detect and measure the physical and chemical characteristics of a population of cells or particles.

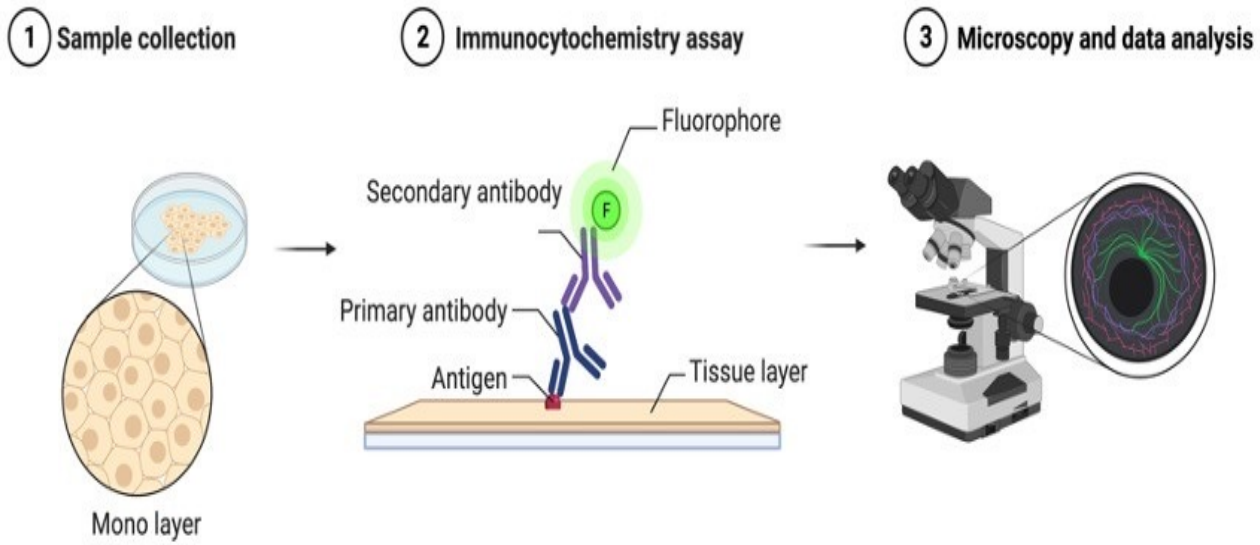
Immunocytochemistry

1

Detection of surface antigens (markers) on isolated cells

2

The detection is based on specific antigen-antibody binding (immunoreactions).



ICC in Diagnostic Cytology Applications

Tumour Diagnosis & Classification

Predictor Markers

Target Therapy

Immunocytochemistry Fixation

95% isopropyl alcohol

Buffered formalin

Formol-acetone

Mixture of ethanol & formalin

Prolonged fixation (weeks/months) in formalin may result in antigenic loss

Prolonged fixation in alcohol-based fixatives is not a major problem

Main methodical steps of immunocytochemistry

1.Cell fixation

2.Antigen unmasking

3.Blocking

4.Selection of appropriate detection signals

5.Parallel detection of more antigens

6.Background staining

7.Controls

Commonly Used Markers In Effusions

Mesothelial Markers	Adenocarcinoma Markers	Breast cytology
Calretinin	MOC-31	ER
WT-1	BerEP4	Mammaglobin
D2-40	mCEA	GATA 3
HBME-1	LeuM1/CD15	E cadherin
CK5/6	B72.3/TAG	P120 catenin

References

Cytopathology (Fundamentals of Biomedical Science), Oxford Oxford University Press [2018]

Modern Techniques in Cytopathology, Bui, Marilyn M. (Tampa, FL)
Pantanowitz, Liron (Pittsburgh, PA),2020Springer

Atlas of Non-Gynecologic Cytology (Atlas of Anatomic Pathology) 1st ed. Jing, Xin, Siddiqui, Momin T., Li, Qing Kay (Eds.),2018 Edition. Springer

Basic and Advanced Laboratory Techniques in Histopathology and Cytology, Dey, Pranab. 1st ed. 2018 Edition, Springer

Cytology: Diagnostic Principles and Clinical Correlates 5th Edition. Cytology: Diagnostic Principles and Clinical Correlates 5th Edition. Elsevier