Fixation of Cytology Specimens-I

BY:

Dr. Aparna Khandelwal

Assistant Professor, Pathology

MMIMSR

- Proper sample collection, fixation and processing are the important components of routine laboratory technique in cytology.
- Unlike histopathology, the specimen collection is widely variable in cytology.
- Different types of fixatives are used in various cytology samples.

- Moreover, the processing of the cytology is widely different in cytology samples.
- Ethyl alcohol (95%) is the most commonly used fixative in cytology.
- The cytology samples are processed commonly by direct smear, centrifugation, cytocentrifugation, liquid-based preparation, and cell block

Fixation

- fixation means :

  - prevention of degeneration of cells and tissue

  - preservation of cells as close as possible to the living state

- For a specific periods of time

Aims of Fixation :

1. To prevent autolysis & putrefaction of the cell.
2. To prevent the loss of celluar constituents
3. It should penetrate evenly and rapidly.
4. To increase tissue consistency, in order to facilitate their going through the other steps of the technique – especially slicing

Aims of fixation :

5. Increase the optical density
6. Should not cause shrinkage or swelling of the cells
7. Must not react with the receptor sites & thus must not interefere with the staining procedure.
8. It must be cheap and easily available.

Ideal fixative

- Penetrate cells rapidly
- Minimize cell shrinkage
- Maintain morphologic integrity
- Deactivate autolytic enzymes
- Replace cellular water

Ideal fixative

- Help diffusion of dyes across cell boundaries
- Help cells adhere to a glass surface
- Provide consistent results over time
- Fixation artefacts should not be there
- Preserves cellular constituents

Ideal fixative

- Cheap
- Nontoxic
- Nonflamable
- Nonirritant
- Kill bacteria

- **Effects of Fixation**
- Sudden death of Tissues but preserves the structure.
- Coagulation of proteins
- Prevents diffusion of substances
- Hardens tissues –prevents from shrinkage or swelling
- Changes refractive index
- Facilitates action of dyes in staining
- Prevents autolysis and bacterial putrefaction

Classification of Fixatives:-

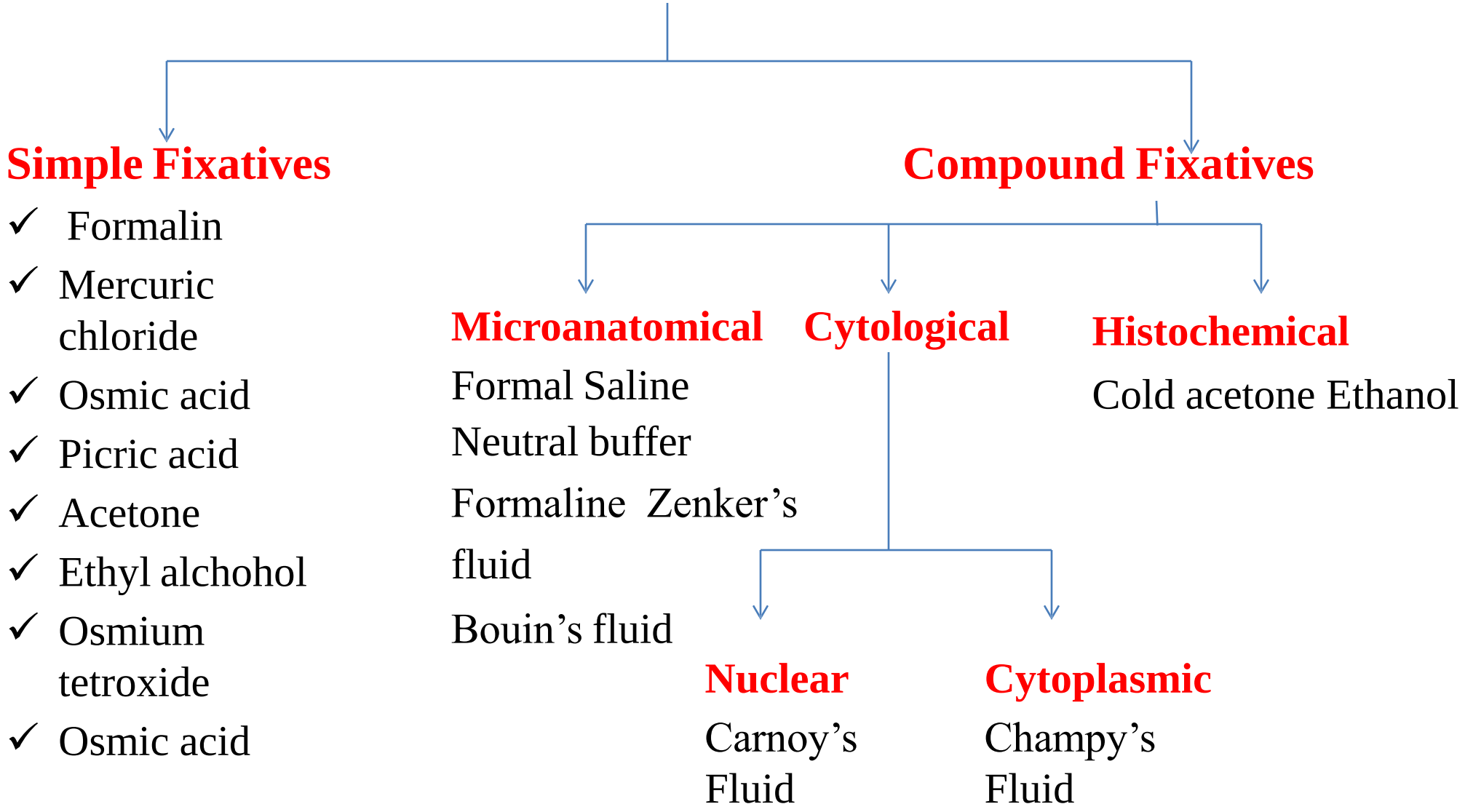
- **1) Physical fixative**

✓ Heat

✓ Freezing

- **2) Chemical fixatives**

Chemical Fixatives



- **Simple fixatives**

These are the individual substance having fixative action on tissue

- **Compound Fixatives**

In order to get proper and nearly ideal fixative actions, simple fixatives are mixed together called compound fixative

Compound Fixatives

Microanatomical fixatives:

- These are used to preserve the anatomy of the tissue.

Cytological fixatives:

- These are used to fix intracellular structures.

Histochemical fixatives :

- These are used to demonstrate the chemical constituents of the cell.

• Cytological Fixatives

Nuclear fixatives :

Carnoy's Fluid

Clarke's Fluid

Newcomer's Fluid

Flemming's Fluid

- **Cytoplasmic Fixatives :**
- Champy's Fluid
- Regaud's Fluid

Carnoy's fluid –

- Ethanol - 60ml
- Chloroform - 30ml
- Glacial acetic acid - 10ml

Champy’s fluid –

- Methanol, absolute - 60.0 ml
- Chloroform - 30.0 ml
- Glacial acetic acid - 10.0 ml

Fixation in cytology

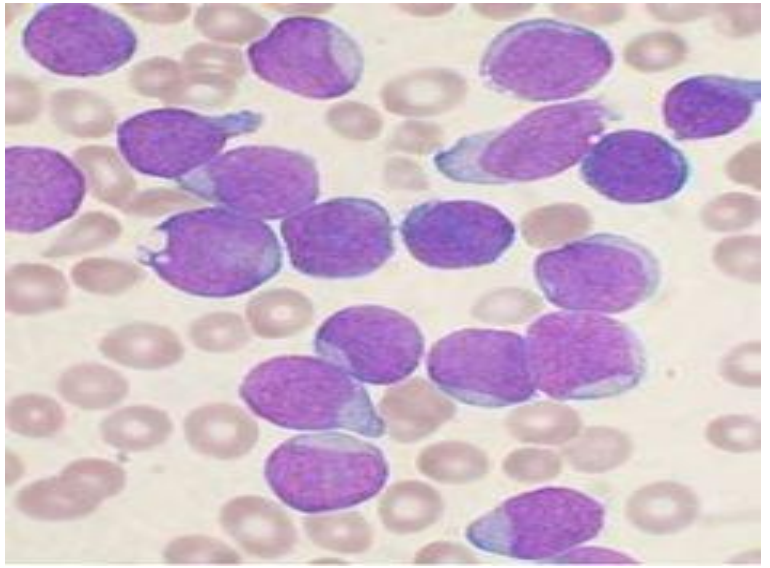
- No fixative is needed for specimen if specimen is immediately delivered or can be refrigerated
- If fixative is required, smears must be fixed immediately – regardless of the method of fixation
- Air drying artifact renders slides unsuitable for interpretation

FIXATION METHODS

- Air drying
- Wet Fixation
- Wet Fixation with Air Drying
- Spray Fixation
- Lysing Fixation for Bloody Samples
- Liquid-based Fixation for Papanicolaou Tests

Dry fixation:

- The slide is dry by air quickly after the material is spread on the slide.
- Followed by hematological stains like Wright–Giemsa, Diff-Quik, or May–Grünwald–Giemsa staining procedures.



Fixation Methods

- Less desirable methods
  - Limited options for specimen manipulation
- Alcohol
  - Can be used if delay in specimen transport or specimen cannot be refrigerated

- Formalin as fixative in cytology
  - Least desirable
  - Fixes not only cells but proteins, debris, blood

Wet fixation:

- The process of submerging of freshly prepared smears immediately in a liquid fixative is called wet fixation.
- This is the ideal method for fixing all gynecological and non-gynecological smears

Wet Fixation of smears

- Smears are fixed either by spraying with fixative or immersing slide in liquid fixative
- Slide should remain in fixative for minimum of 15-30 minutes, not more than 1 week

- **Liquid fixatives**
  - Ethanol
    - Most common is 95%
  - Methanol – less cell shrinkage, cytogenetics,
    - 100% methanol comparable to 95% ethanol
  - Coating fixatives
    - Must soak slides in 95% alcohol for up to 10 minutes to remove coating fixative and then stain slides
  - Methanol/diethyl ether (not used)
  - Acetone

Spray Fixatives

- Spray fixatives are commercially available
- Usually contain ethanol or isopropyl alcohol with carbowax (polyethylene glycol)
- After alcohol evaporates, cells are left coated with carbowax, which prevents cell shrinkage
- Other spray fixatives include commercially available aerosol fixatives
  - Hair spray – not recommended

Wet fixation

- **A) Routine fixative**
- **B) Coating fixative**
- **C) Special purpose fixative**

(A) Routine Fixative

- Mainly alcohol based fiaxtives
- All alcohol fixatives should be discarded or filtered (Whatman No: I filter paper) after each use.
- Any of the following alcohols can be used:

Cytolological Fixatives:-

- Alcohols:- Specifically recommended for cytological preparation

(1)95% Ethanol/Ethyl alcohol

(2)95 %Rectified Spirit

(3)100% Methanol

(4) Isopropyl alcohol/propanol

(5) Alcohol Ether (1:1)

Wet fixation

95% Ethyl Alcohol (Ethanol)	• Ideal fixative recommended • Dehydrating agent • Desired amount of cell contraction • Yield optimal chromatin detail characteristics
Ether alcohol mixture	•Ether and 95% ethyl alcohol • 1 : 1 • excellent fixative, but ether - **Fire Hazard**
100% Methanol	• produces less shrinkage than ethanol • more expensive
80% Propanol and Isopropanol	•cause slightly more cell shrinkage
Denatured alcohol	• 90 parts **of 95% ethanol** + 5 parts of **100% methanol** + 5 parts of **100% isopropanol.**

Time of wet Fixation

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


Thank You
www.DesktopBackground.or