Fixation of Cytology Specimens II

BY:

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- Proper sample collection, fixation and processing are the important components of routine laboratory technique in cytology.
- Different types of fixatives are used in various cytology samples.
- Ethyl alcohol (95%) is the most commonly used fixative in cytology.
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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

FIXATION METHODS

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

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

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

B. Coating fixatives

- Coating fixatives are substitutes for wet fixatives.
- They are either aerosols applied by spraying the cellular samples or a liquid base, which is dropped onto the slide.
- They are composed of an alcohol base, which fixes the cells and wax like substance, which forms a thin protective coating over the cells e.g. Carbowax (Polyethylene Glycol) fixative.

Coating fixatives

- Diaphine fixative Spray coating fixative (Hairspray) with high alcohol content and a minimum of lanolin or oil is also an effective fixative.
- Aerosols or liquid base

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

- **Carbowax** (Polyethylene Glycol) fixative.

- Have practical value in situations where smears have to be send to a distant cytology laboratory for evaluation
- Not recommended for smears prepared from fluids within the laboratory.
- Not recommended for bloody smears because they cause clumping of erythrocytes

Coating fixatives

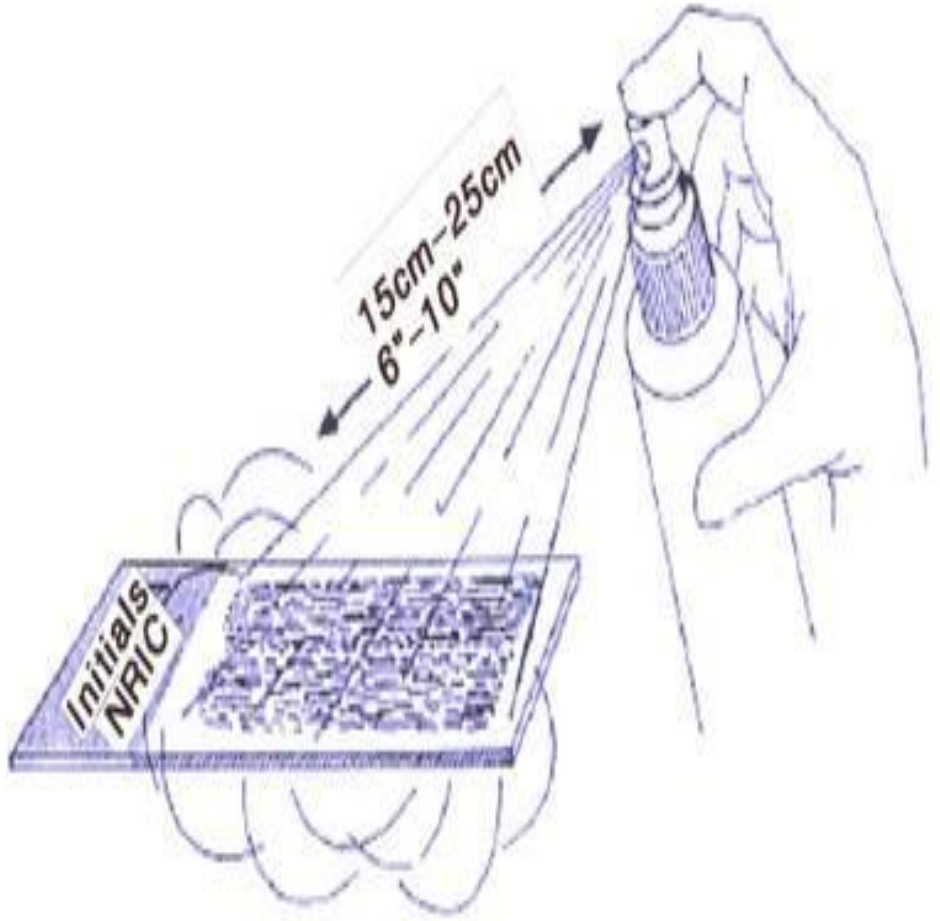
- Instructions for applying the coating fixative should be followed carefully.
- Cans should be shaken well prior to each use to ensure optimal dispersal and adequate fixation.
- Should be applied immediately to fresh smears.

Coating fixatives

- The distance from which the slides are sprayed with an aerosol fixative affects the quality of the cytologic detail.
- The optimal distance differs with the brand of fixative used
- A distance of 10-12 inches (25-30cm) is considered optimal.

- Waxes and oils from hair spray fixative alter staining reactions if they are not adequately removed.
- Prior to staining, the slides have to be **kept overnight in 95% alcohol** for removal of the coating fixative.

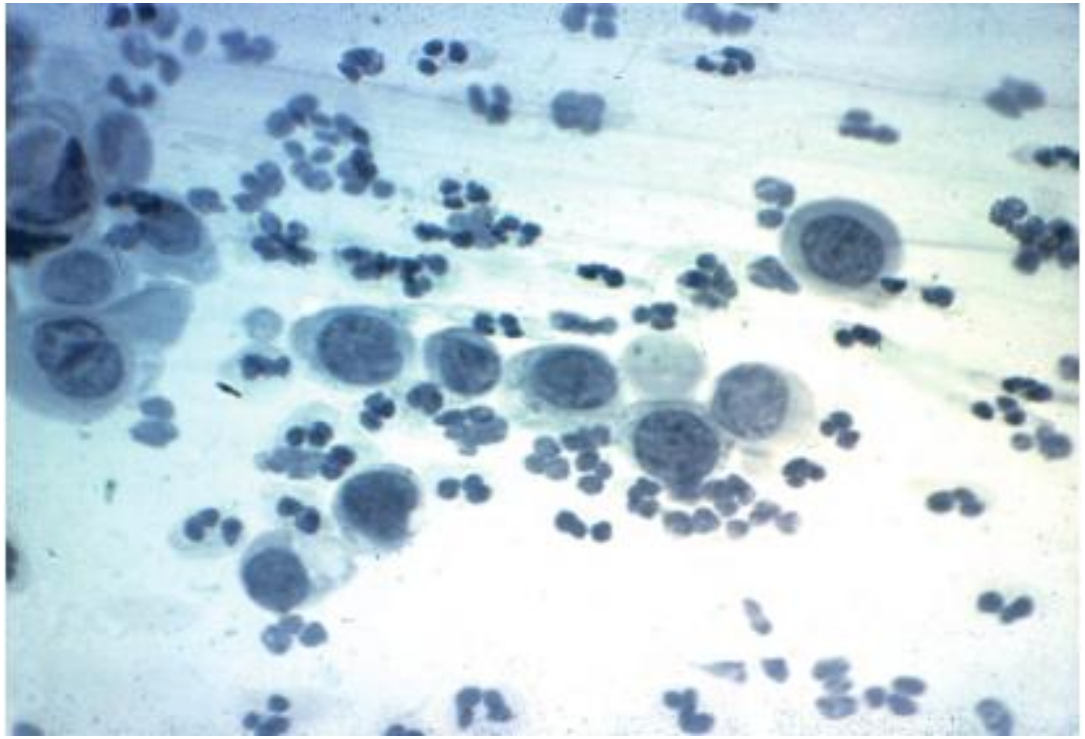
The 95% ethyl alcohol used for washing off the coating fixative should be filtered or changed at least once each day, the number of times depending on the number of slides that are washed



if the carbowax is not removed completely, Nuclei will then appear foggy and lack chromatin detail and the cytoplasm may exhibit a pale blue color

carbowax not removed
Lack of chromatin details and hazy a ppearance of cell

HSIL, Pap Hp



Special-Purpose Fixatives

- **Neutral Buffered Formaldehyde Solution** and **Bouin's Solution** are used for preserving nuclear features in small samples, such as **cell blocks.**
- **Saccomanno's Fixative**
- **Carnoy’s fixative**
- **AAF Fixative**

Saccomano’s Fixative

- **(**50% alcohol and approximately 2% Carbowax)
- Carbowax infiltrates and occupies submicroscopic spaces, preventing cell collapse and thus protects the cells during air drying.
- Cells adhere well to glass slides as a consequence of air drying.
- This fixative was first used by Saccomanno for prefixation of sputum but can be used for fluid specimens from other sites.

Carnoy's fixative

- special purpose fixative for **haemorrhagic samples**
- Absolute ethanol, Chloroform and Glacial acetic acid (6 : 3 : 1)
- Acetic acid in the fixative haemolyses the red blood cells.
- an excellent **nuclear fixative** as well as a preservative for glycogen
- but results in considerable shrinkage of cells and tends to produce over staining in hematoxylin

- Over fixing in Carnoy’s also results in loss of chromatin material.
- Carnoy’s fixative must be prepared fresh when needed and discarded after each use.
- It loses its effectiveness on long standing, and chloroform can react with acetic acid to form hydrochloric acid

Modified Carnoy's

95% ethanol	Chloroform	Glacial acetic acid
7	2.5	0.5
6	3	1
6		1

AAF Fixative

- This is the ideal fixative used for **cellblock** preparation of fluid specimens

AAF Fixative:

- 95% Ethanol 34ml
- Formalin 4ml
- Glacial acetic acid 2ml

Other solutions used to lyse red blood cells:

- **Clarke's solution**: absolute ethanol, glacial acetic acid (3 : 1)
- One drop of conc **HCl** per 500 mL of 95% ethanol
- 10% **Glacial acetic acid** (this is followed by placing the slide in 95% ethanol)
- Commercially available fixatives such as

CytoRich Red

Transport of unstained smears

Glycerine method :

- Smears are first fixed in 95% ethanol for 12 minutes and removed.
- Two drops of glycerine are placed on smears and covered with a clean glass slide.

- This may be wrapped in wax paper and sent to the laboratory in a suitable container.
- Coating fixative such as carbowax fixative and spray coating fixative can be used primarily to facilitate transport of smears

Prefixation of Cytological material

- Prefixation may preserve some specimens for days without deterioration of cells.
- Some of the disadvantages of pre-fixation are precipitation/coagulation of proteins, hardening of cells in spherical shapes and condensation of chromatin.

- The coagulation of proteins may interfere with the adherence of cells to glass slides.
- It also ‘rounds up’ the cells - causes the cells to gather together into tight clusters making stain absorption and interpretation difficult.
- Albuminized slides should be used to prepare smears from prefixed sample.

The most common solutions used for prefixation are

- Ethyl alcohol
- Sacomanno’s fixative (50% alcohol with 2% Carbovax 1540)
- Mucolexx (A commercial mucoliquifiing preservative for the collection of mucoid and fluid specimens)

Rehydration of Air Dried Smears

- Unfixed, air-dried gynaecological smears received from **peripheral areas** can be used for **Papanicolaou staining** by rehydration method
- The simplest rehydration technique is to place **air dried cytological specimens in:**

50 % aqueous solution of glycerine for 3min

2 rinses in 95% ethyl alcohol

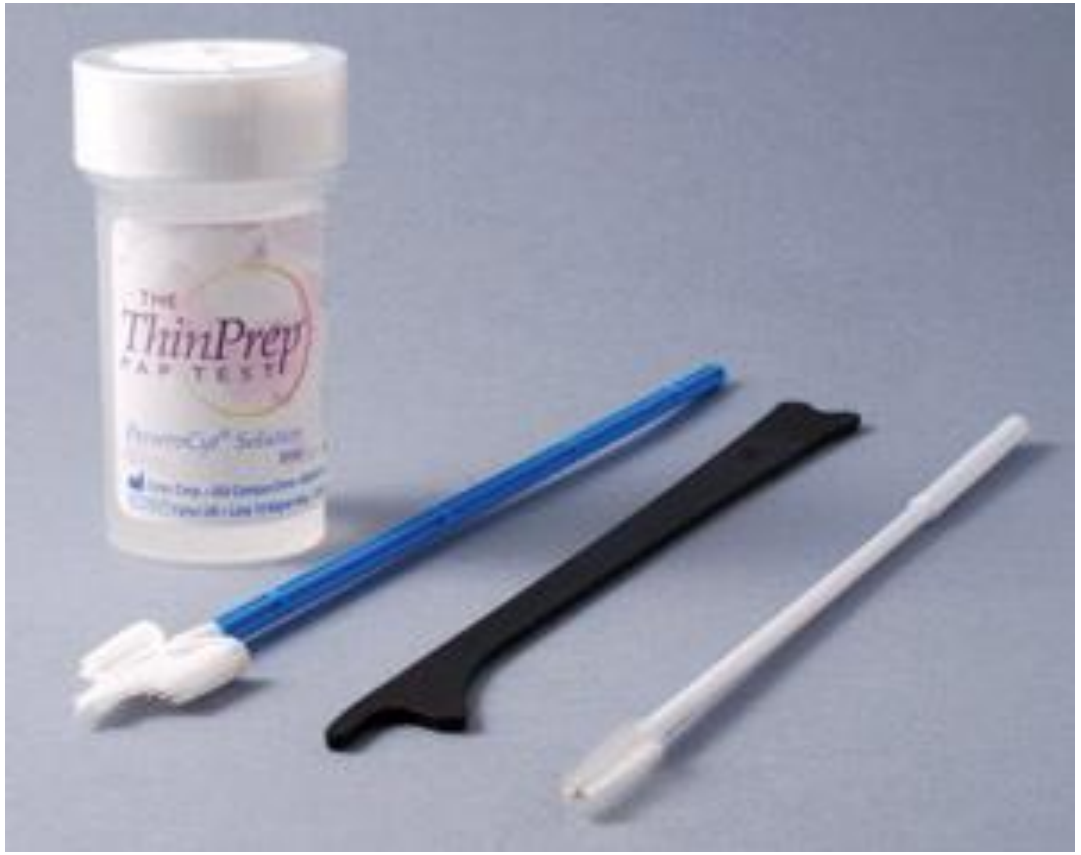
Routine Pap staining

Liquid-based cytology (LBC)

- Cytology (the study of cells) through a liquid medium
- Cells are collected from cervix (or any other site) are placed **directly into liquid preservative**, rather than transferred to slide.
- Sample is processed and resultant thin smear is easy to screen

Liquid-based Fixation

- the sample is collected to vial contains fixative solutions mostly methanol.
- This type of fixative is suitable for transport of specimen to other place and can be stored up to 7 days or more.



STORAGE REQUIREMENTS

The following guideline is recommended for medical care locations:

1. Central storage locations are to be non-patient care areas that are properly ventilated.
2. Minimize the number of specimen containers in a location.

3. The quantity stored in these locations should be limited to a one-month’s supply for any size

4. The specimen containers should be kept in the original shipping container, if possible.

5. A label is to be placed on the shelf or cabinet to identify storage location.

The following steps are to be taken when chemical spill:

1. Inform others in the area of the minor spill.

2. Wearing the appropriate personal protective equipment to prevent exposure (minimum of gloves and an outer garment)

3. Absorb the spilled material with tissue paper.

4. Place the tissue paper into a labeled hazardous waste container

5. Wash the contaminated area two times with some soap and water.

6. Dry the area with tissue paper.

7. Seal the container to minimize the release of chemicals and fixatives.


Thanks
THANK YOU EVERYONE!