Gynecological cytology

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Learning Objectives

1. **Define Gynecological Cytology and explain its importance in medicine.**
2. **Explain Cytology Gynecology Test Requisition**
3. **Explain different PAP Smear Collecting devices and Collecting Techniques**
4. **Discuss PAP Smear Fixation method**
5. **Describe PAP stain method**
6. **Other Gynecological Cytology Tests**

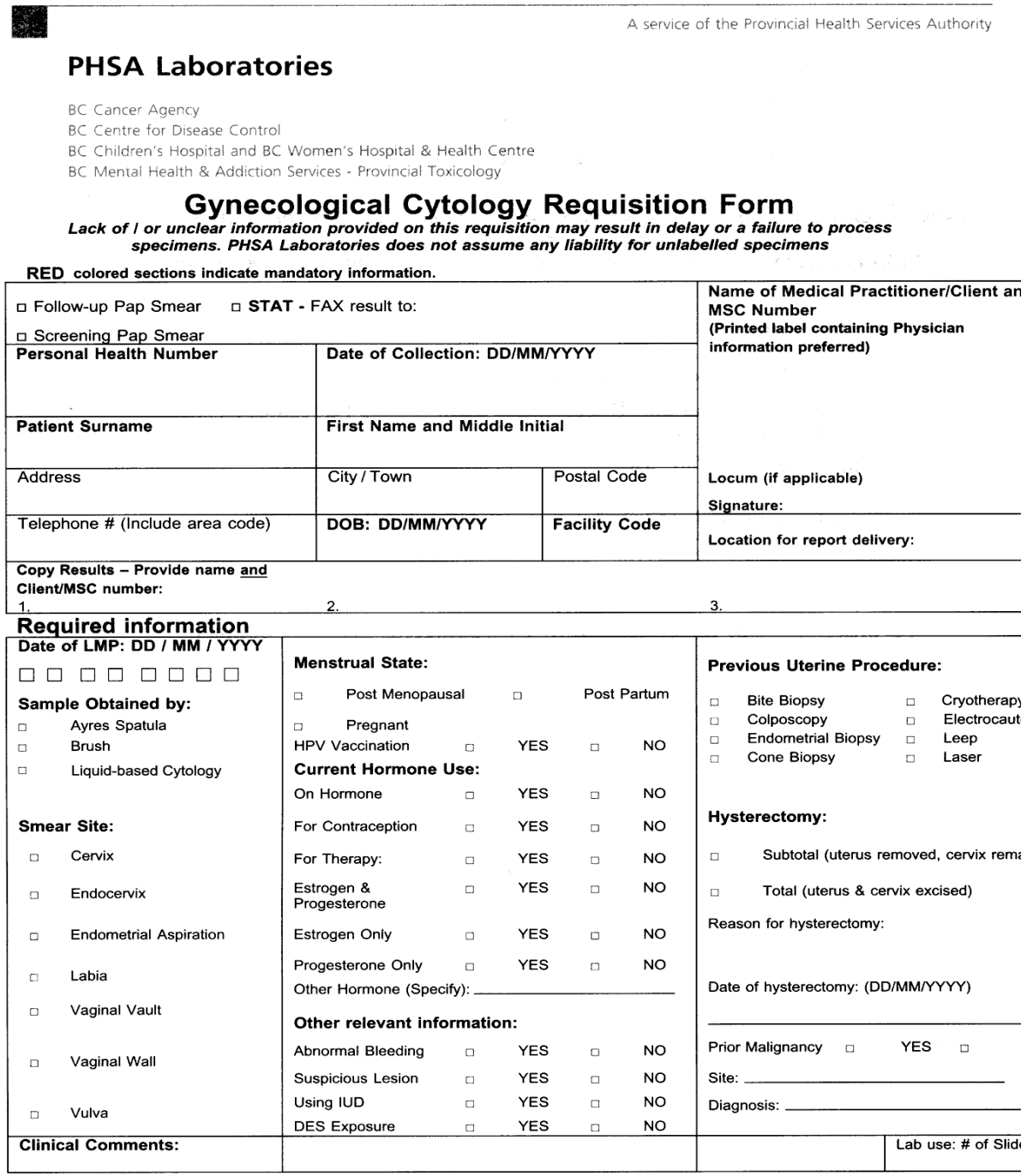
Gynecological Cytology Definition and its Importance

- **Definition:** Gynecological cytology examines cells from the female reproductive tract, primarily the cervix, to detect abnormalities, including precancerous changes and infections.

- **Importance:** This crucial medical practice, epitomized by the Pap smear, is vital for early detection and prevention of cervical cancer.

Cytology Gynecology Test Requisition

- **The requisition should be filled out completely and include:**
- Patient identification (Name & Hospital number)
- Requested test
- Specimen source
- Date and time of specimen collection
- Clinical information



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PHSA Laboratories

BC Cancer Agency
BC Centre for Disease Control
BC Children's Hospital and BC Women's Hospital & Health Centre
BC Mental Health & Addiction Services - Provincial Toxicology

Gynecological Cytology Requisition Form

Lack of / or unclear information provided on this requisition may result in delay or a failure to process specimens. PHSA Laboratories does not assume any liability for unlabelled specimens

RED colored sections indicate mandatory information.

☐ Follow-up Pap Smear ☐ **STAT -** FAX result to: ☐ Screening Pap Smear			**Name of Medical Practitioner/Client an MSC Number** **(Printed label containing Physician information preferred)**
Personal Health Number	**Date of Collection: DD/MM/YYYY**		
Patient Surname	**First Name and Middle Initial**		
Address	City / Town	Postal Code	**Locum (if applicable)** **Signature:**
Telephone # (Include area code)	**DOB: DD/MM/YYYY**	**Facility Code**	**Location for report delivery:**

Copy Results – Provide name and Client/MSC number:
1. 2. 3.

Required information

Date of LMP: DD / MM / YYYY
☐☐ ☐☐ ☐☐☐☐

Sample Obtained by:
- ☐ Ayres Spatula
- ☐ Brush
- ☐ Liquid-based Cytology

Smear Site:
- ☐ Cervix
- ☐ Endocervix
- ☐ Endometrial Aspiration
- ☐ Labia
- ☐ Vaginal Vault
- ☐ Vaginal Wall
- ☐ Vulva

Menstrual State:
☐ Post Menopausal ☐ Post Partum
☐ Pregnant

HPV Vaccination ☐ YES ☐ NO

Current Hormone Use:

On Hormone	☐ YES	☐ NO
For Contraception	☐ YES	☐ NO
For Therapy:	☐ YES	☐ NO
Estrogen & Progesterone	☐ YES	☐ NO
Estrogen Only	☐ YES	☐ NO
Progesterone Only	☐ YES	☐ NO

Other Hormone (Specify): _____

Other relevant information:

Abnormal Bleeding	☐ YES	☐ NO
Suspicious Lesion	☐ YES	☐ NO
Using IUD	☐ YES	☐ NO
DES Exposure	☐ YES	☐ NO

Previous Uterine Procedure:

☐ Bite Biopsy	☐ Cryotherapy
☐ Colposcopy	☐ Electrocaut
☐ Endometrial Biopsy	☐ Leep
☐ Cone Biopsy	☐ Laser

Hysterectomy:
- ☐ Subtotal (uterus removed, cervix rema
- ☐ Total (uterus & cervix excised)

Reason for hysterectomy:

Date of hysterectomy: (DD/MM/YYYY)

Prior Malignancy ☐ YES ☐

Site: _____

Diagnosis: _____

Clinical Comments: | Lab use: # of Slid

Collecting Devices

1) Ayre spatula (wooden or plastic):

- **Wooden** spatulas are porous, **only about 20%** of the exfoliated cells which are obtained are transferred to the PAP test slide.

- The **plastic** Ayre spatula has been shown to have a **better transfer** rate.

- Ayre spatula *is the* ***<u>least effective device</u>*** for collecting cells from the endocervix.

Collecting devices

2) A saline moistened, cotton tipped applicator:
has been used with ***<u>moderate success</u>*** to improve the adequacy of the sample.

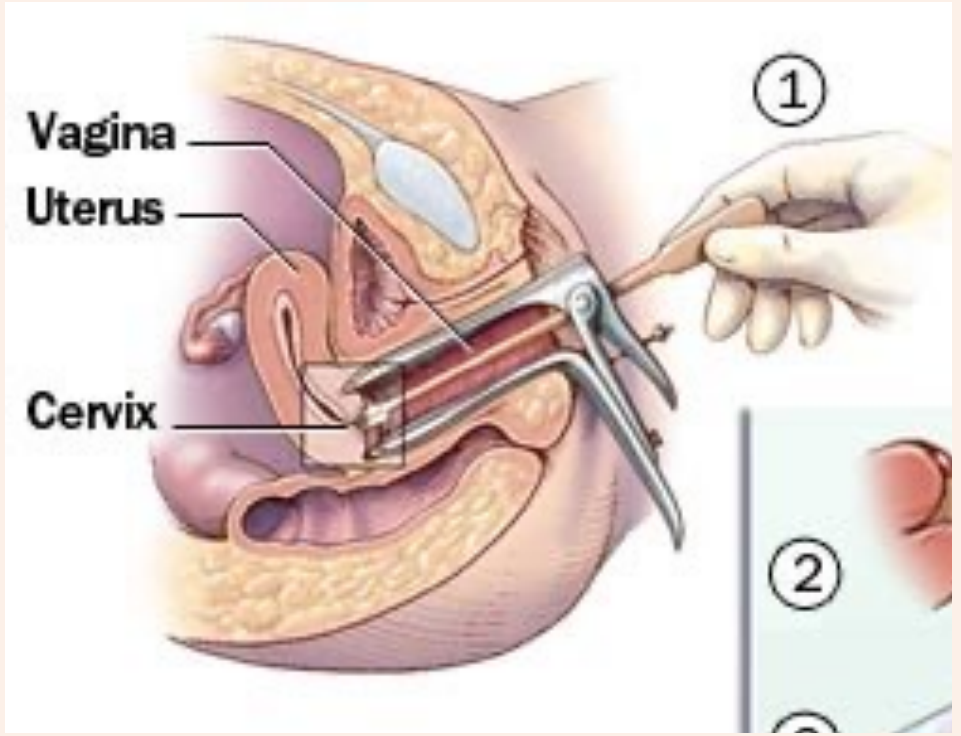
3) Endocervical brush, or the cytobrush:

sampling of the endocervix *<u>has improved the specimen adequacy</u>*.

Sample Collection

Positioning: The patient is positioned on an examination table, with knees bent and feet placed in stirrups to allow access to the cervix.

1.Speculum Insertion: A medical professional inserts a speculum into the vagina to gently widen it, providing a clear view of the cervix.

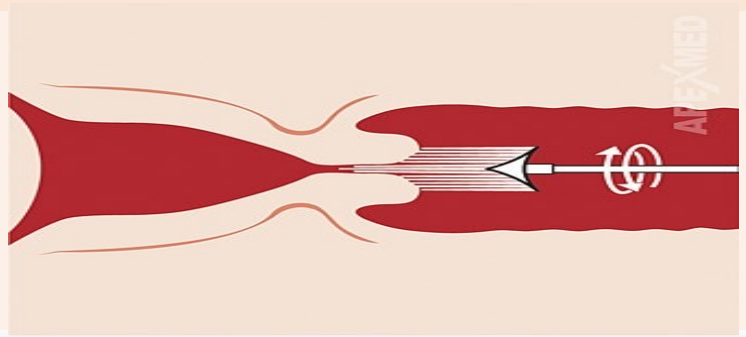


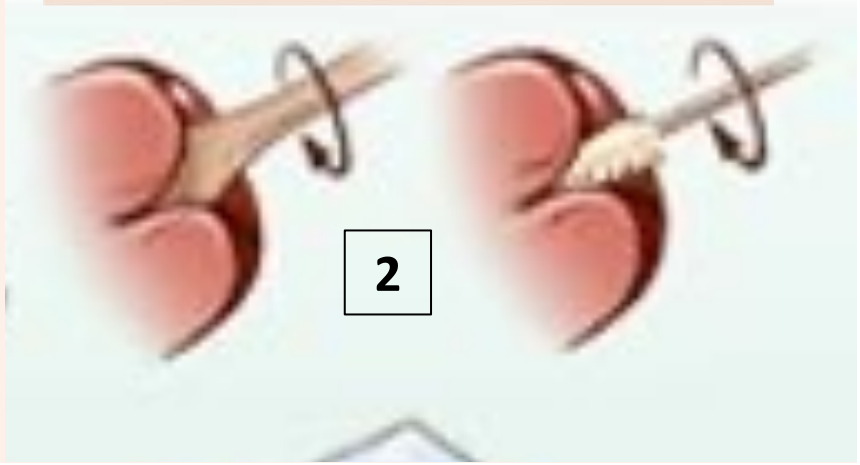
Sample Collection

2. Collection of Cells:

•A spatula or a brush-like device (cytobrush) is used to collect cells from the **surface of the cervix**, mainly focusing on the **transformation zone** (the area where the **outer squamous** cervical cells meet the **inner glandular** endocervical cells). This area is most likely to develop precancerous changes.

•The spatula is rotated around the entire circumference of the cervix to gather squamous cells.
•The brush is then inserted into the cervical canal and rotated to collect endocervical cells.





Sample Collection

Sample Transfer:

3. In conventional cytology, the collected cells are smeared directly onto a glass microscope slide and fixed with a spray or immersion in a fixative.

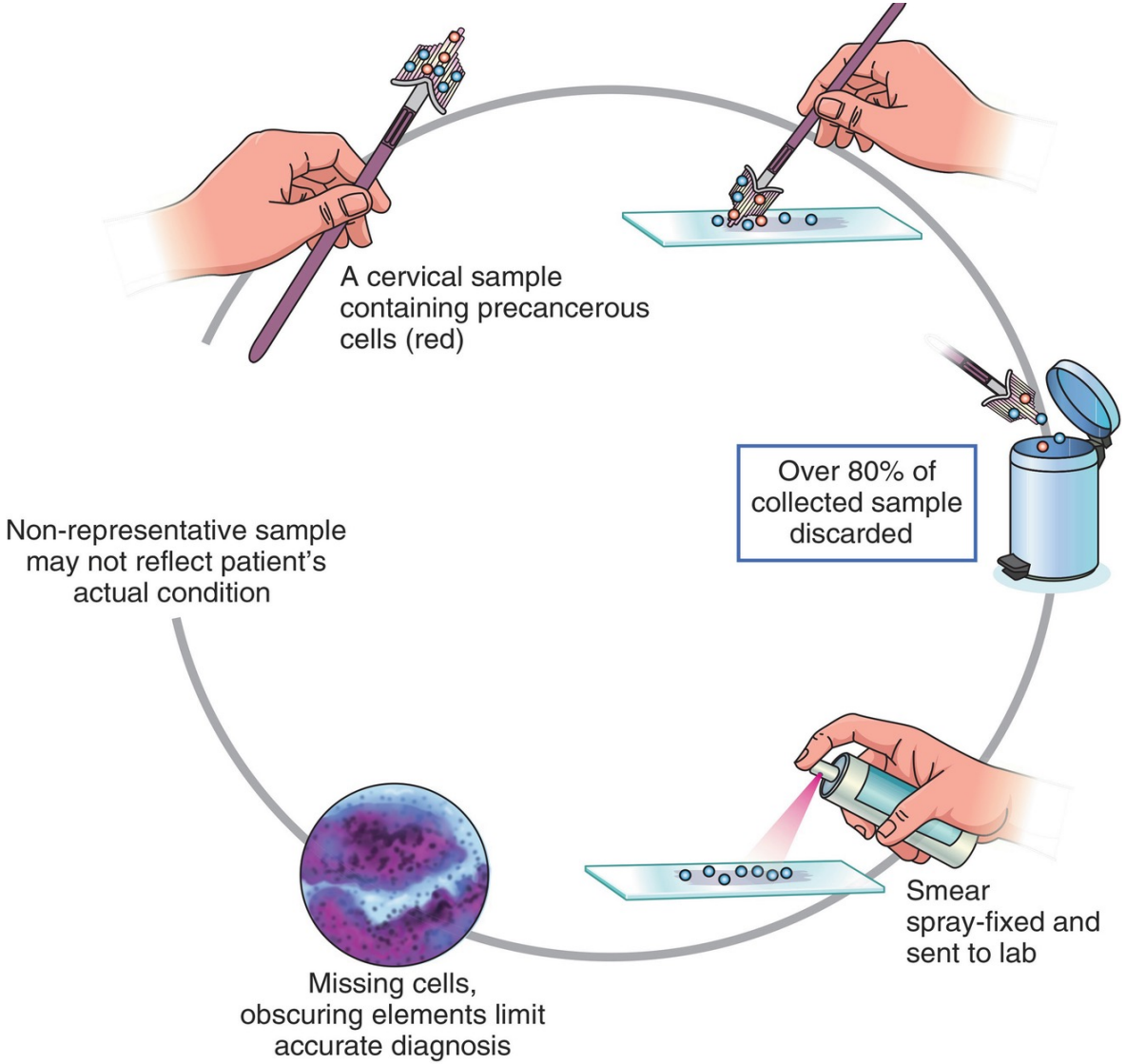
4. The brush is rinsed in a vial containing a preservative solution for liquid-based cytology, suspending the cells.

Sample Collection

5. Labeling and Transport: The sample is labeled with the patient's information and sent to a laboratory for staining and microscopic evaluation.

PAP Smear Collecting Techniques

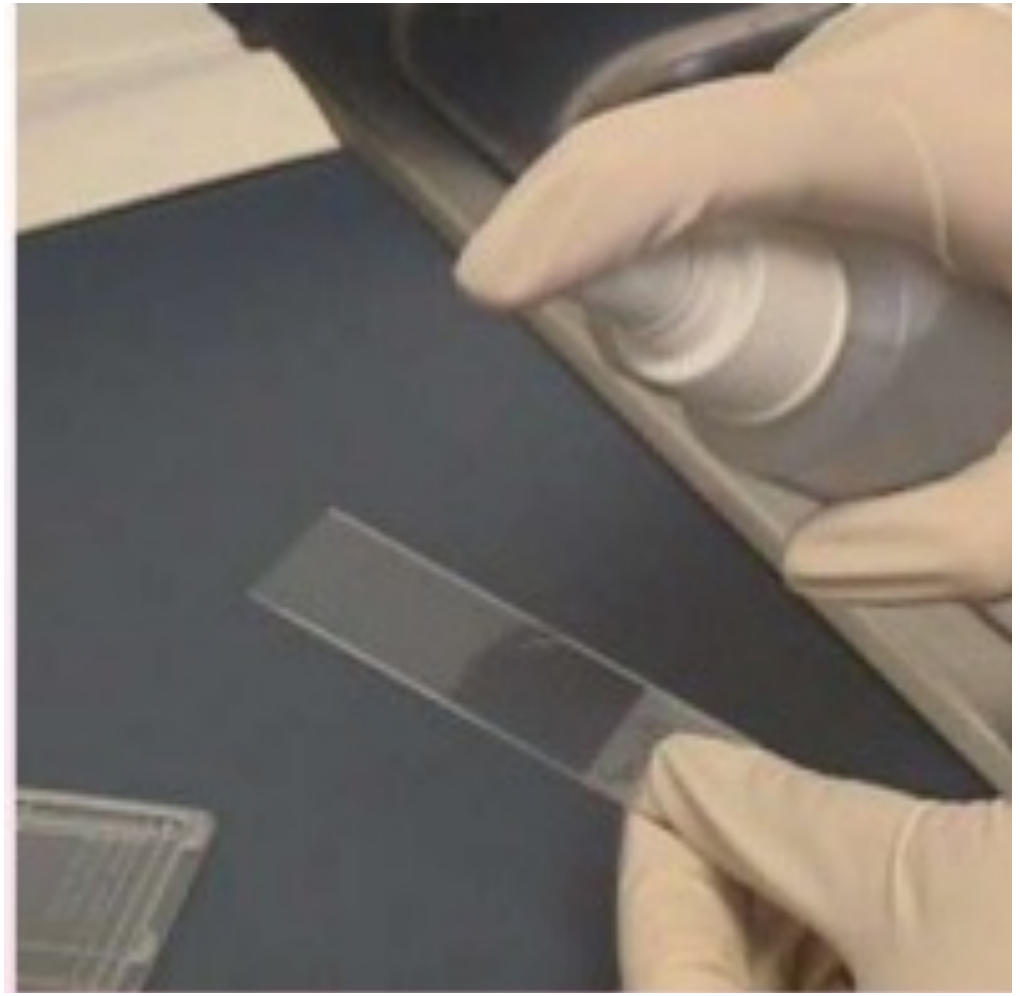
1.Conventional PAP Smear Preparation



In the traditional "smear" method of collecting cervical cells, only a small percentage of collected cells make it onto the slide itself, and abnormal cells may be discarded before they can be reviewed. During review of the slide, cells that have dried out and clumped together can obscure the view of abnormal cells.

Wet Fixation

- ✓Any method that **immerses or sprays cell**
- ✓Commonly Uses: **Ethanol + polyethylene glycol**
- ✓ (is a water-soluble waxy compound that helps prevent cell damage)
- ✓**May be stained with Papanicolaou (PAP) or H&E Stain**



Spray Distance

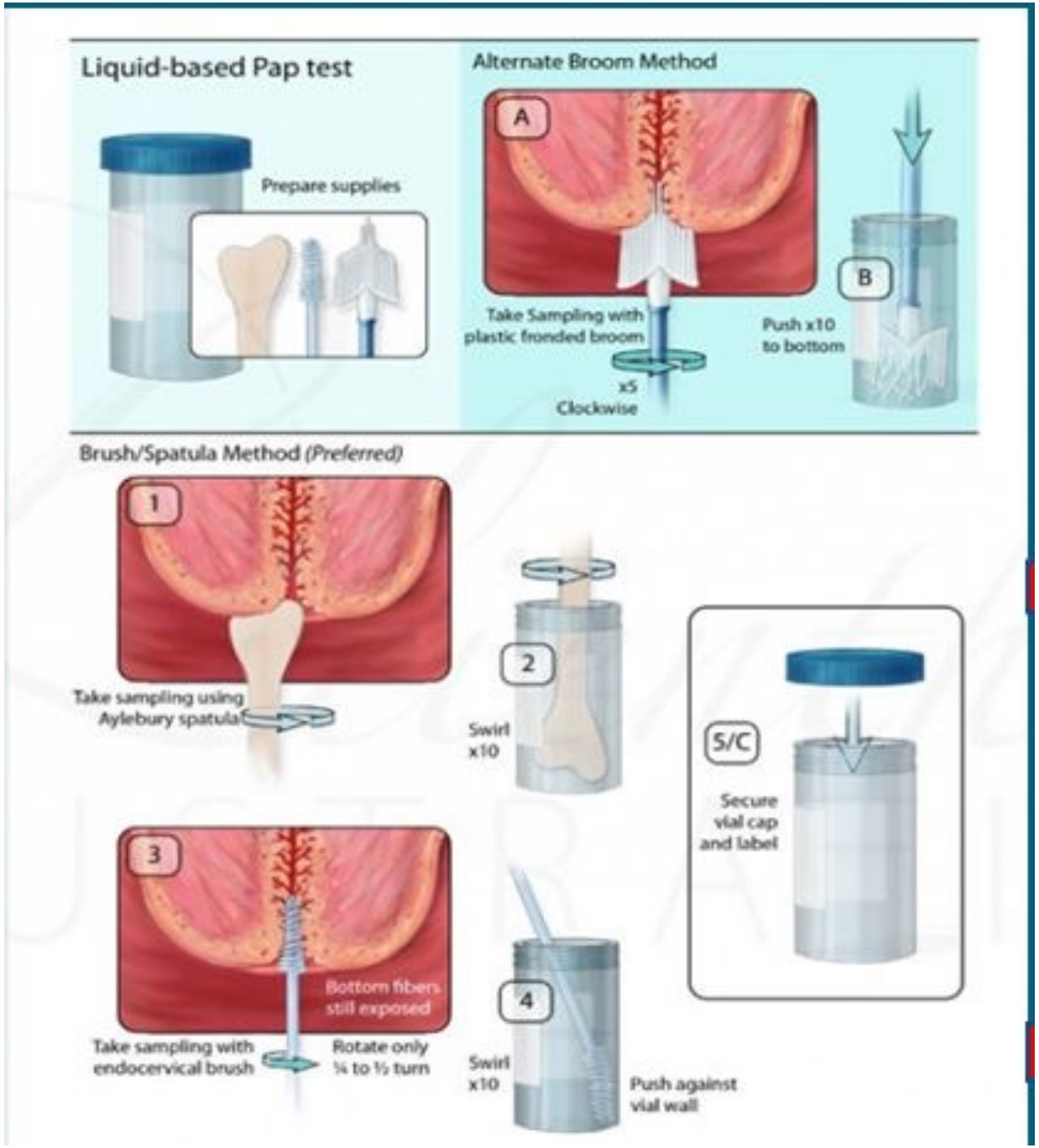
• The **distance** from which the slides are sprayed with an aerosol fixative **affects** the cytology details.

•Please maintain a distance of 15-25cm and an angle of 6-10 degrees.

Before staining, the slides should be soaked overnight in 95% alcohol to remove the coating fixative.

2. ThinPrep PAP Test

- The ThinPrep® Pap test helps healthcare providers detect the presence **of abnormal cervical cells**, and the **HPV** infections that most likely to **lead** to cervical disease



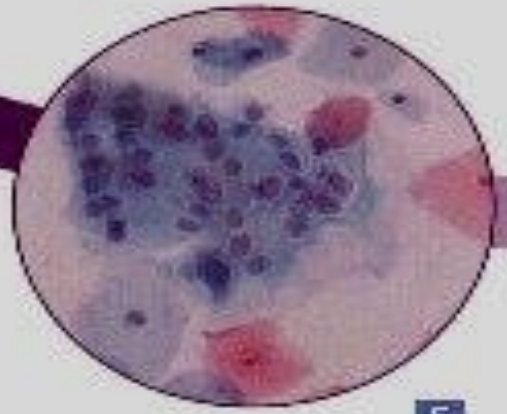
1 Cervical Sample

2 Virtually 100% of collected sample rinsed into ThinPrep® Vial

3 Cells immediately preserved and sent to laboratory

4 Controlled Membrane Transfer disperses, collects and transfers cells

5 Representative thin layer of cells is clear of obscuring elements



INCREASED OPPORTUNITY TO DETECT EARLY SIGNS OF ABNORMALITY

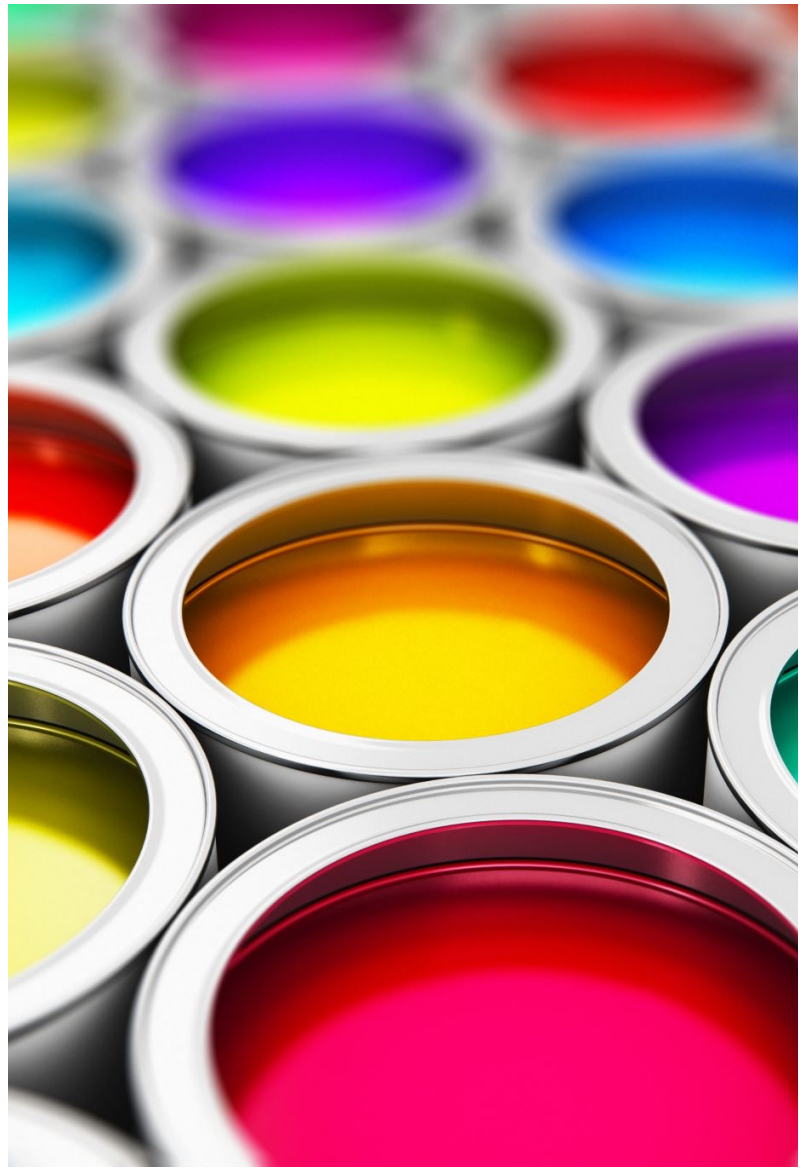
Stains for wet fixed preparations

- **Papanicolaou stain** :

Wider range of colours

- **H & E:**

Simpler faster but less colour differences



Rehydration of Air-Dried Smears

- **Unfixed, air-dried** gynaecological smears received from peripheral areas can be used for Papanicolaou staining by rehydration method

- **The most straightforward rehydration technique is to place air-dried cytological specimens:**

I. 50 % aqueous solution of glycerine
II. Two rinses in 95% ethyl alcohol
III. Pap staining

Papanicolaou Staining (PAP)

- Named after **Dr. George N. Papanicolaou**
- **Polychrome** staining reaction
- Display the many variations of cellular morphology showing degree of **cellular maturity** and **metabolic activity**.

PAP Stain Principles

Hydration and Dehydration:

– **Hydration** prepares the cell sample for uptake of the **nuclear dye**.

– **Dehydration** prepares the cell sample for uptake of the **counterstains**.

Dehydration and clearing solutions result in cellular transparency and prepare the cell sample for the final steps.

PAP Stain

- **Nuclear staining: Hematoxylin**
- **Two cytoplasmic counter staining:**

(1) **Orange G** - (OG)-6, OG-5 and OG-8 is an acidic dye, stains keratin a bright, intense orange.

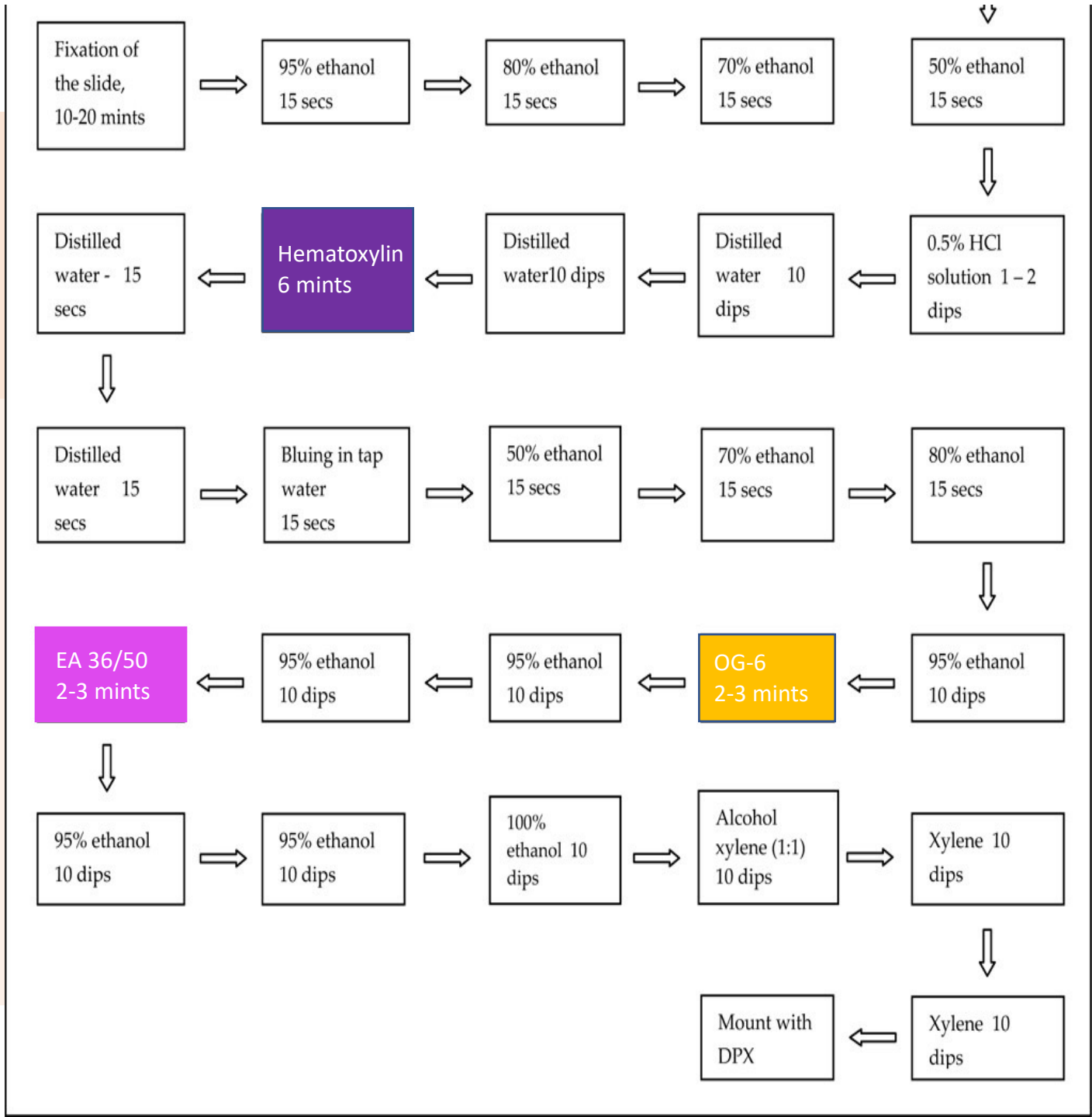
(2) **Eosin Azure** (EA) - EA-36,

EA-50 and EA-65 including

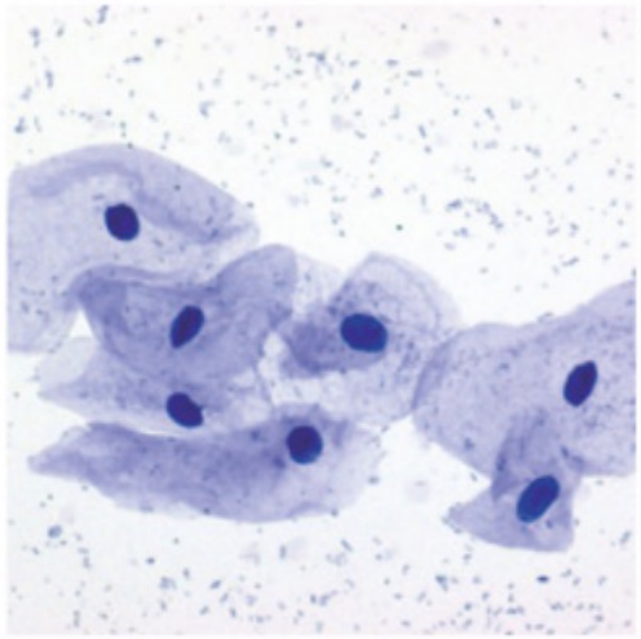
three stains:

- –Eosin Y
- –Light Green
- –Bismarck brown Y

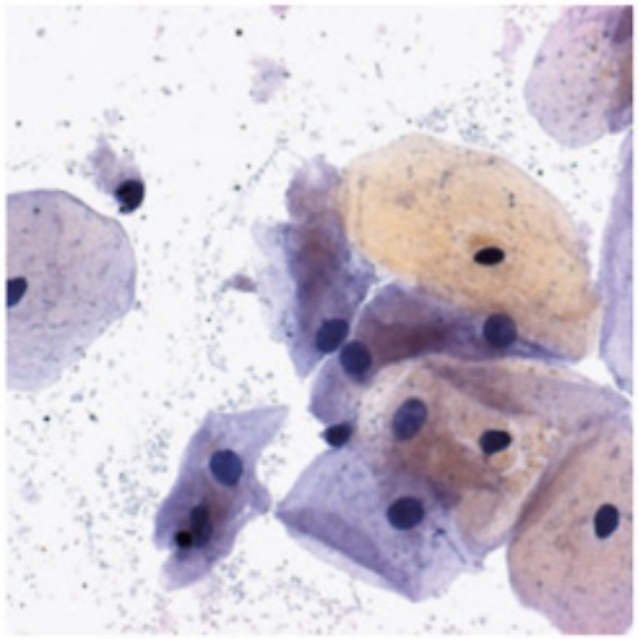
Conventional Pap staining procedure.



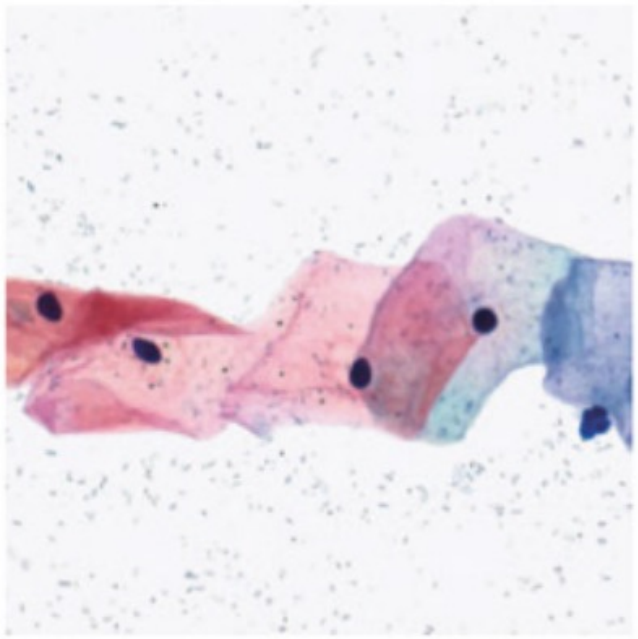
Polychromatic PAP stain



Hematoxylin stain >>>

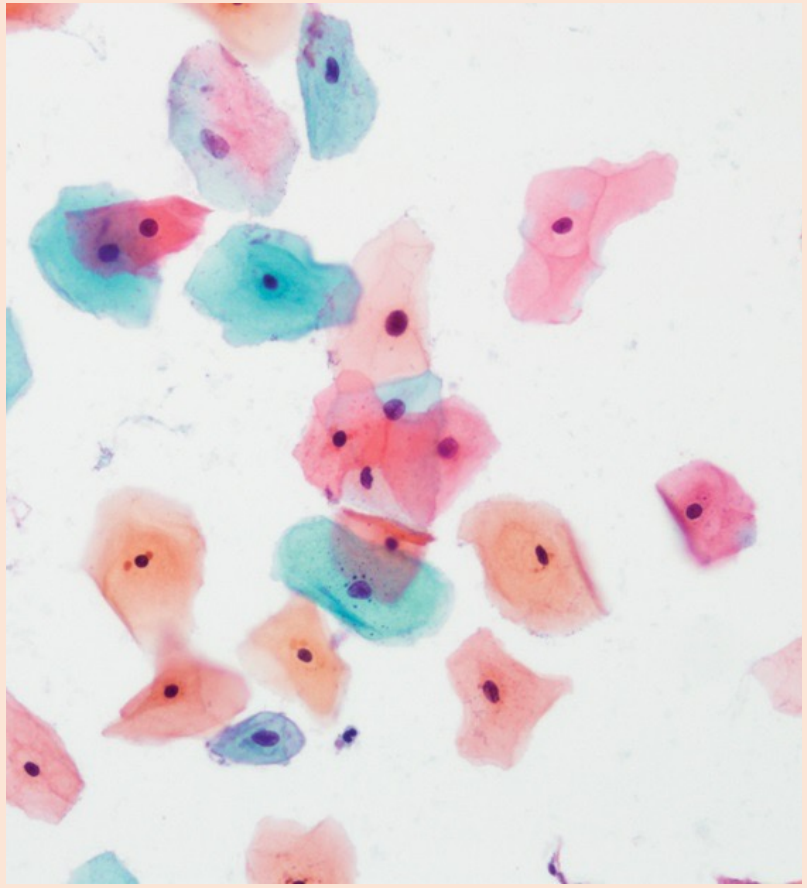


Orange Stain >>>



EA Polychromatic Stain

PAP Smear



- Stained specimen has colours from the entire spectrum!

red, orange, yellow, green, blue, and violet.

- Cell nuclei are distinct and **blue to black**.

- Cells with high content of keratin are yellow, glycogen stains yellow as well.

- **In cervical smears:** Superficial cells are orange to pink, and intermediate and parabasal cells are turquoise green to blue.

Ultrafast Papanicolaou Stain

Fast 90 seconds

1.Smeared on a slide

2.Allowed to air dry

3.Placed in normal saline

4.Fixed in a mixture of 4%formaldehyde and 65% ethanol

5.Stained with Richard Allan Hematoxylin 2 and Cytostain

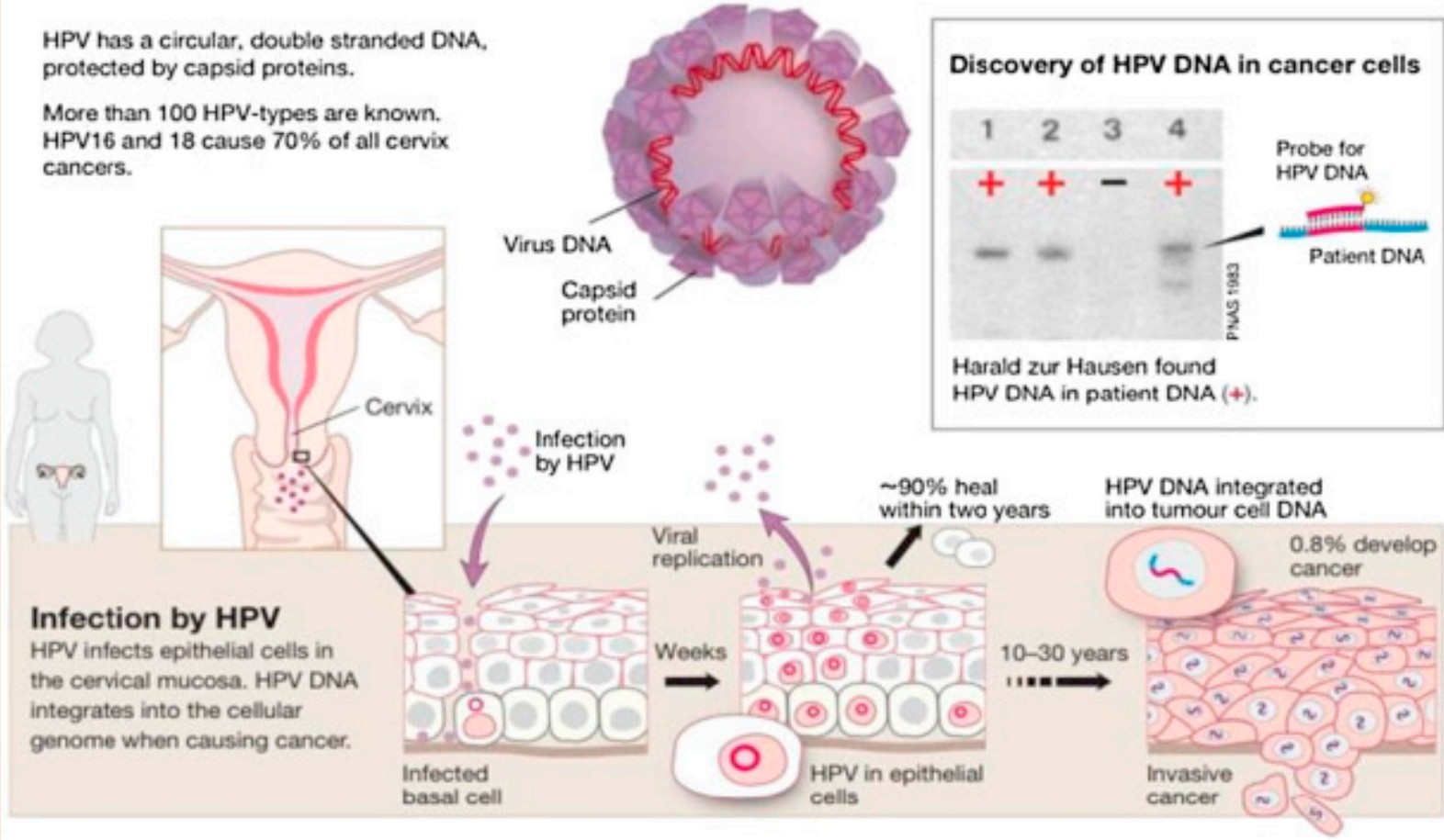
Factors affecting PAP staining

- Type of fixatives
- No. Of slides in each dye
- Age of dyes
- Moisture and humidity
- Quality of cell sample
- The presence or absence of inflammatory cell changes
- Length of staining time

Other Gynecological Cytology Tests:

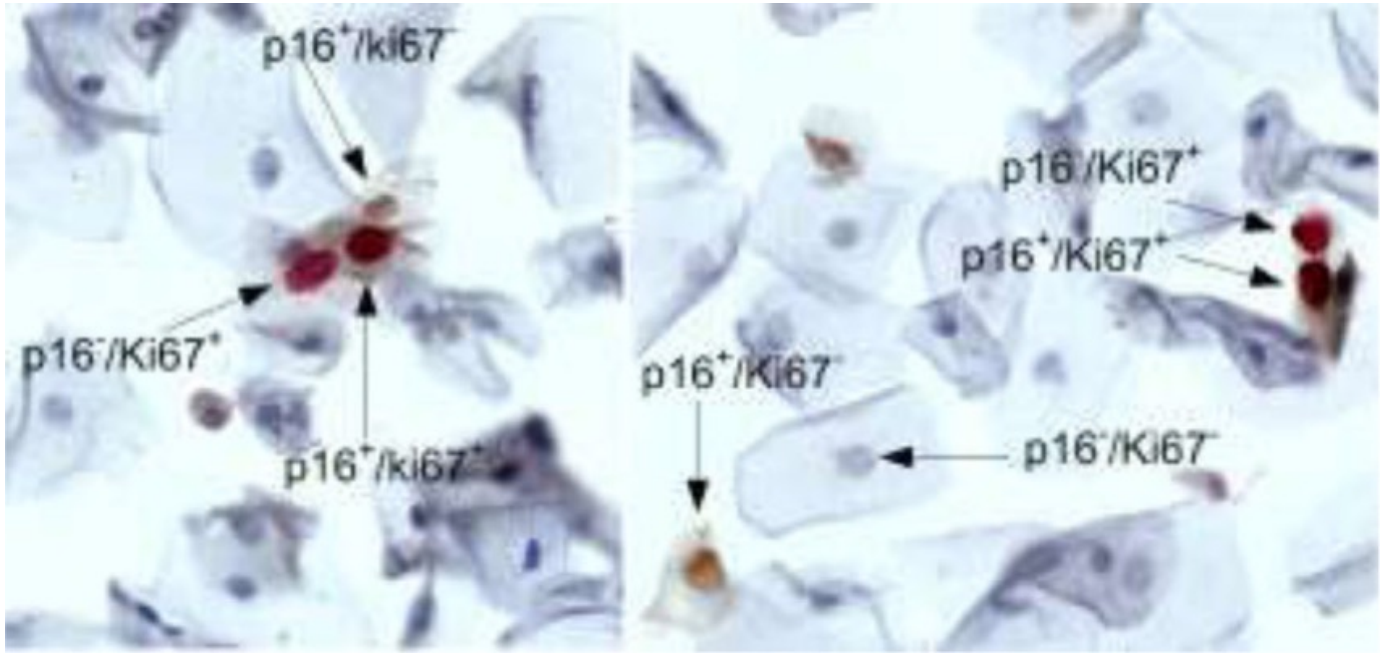
Human papillomaviruses (HPV) and Cytology

- HPV DNA testing alongside cytology enhances cervical cancer screening by identifying high-risk HPV strains linked to cancer development.



Other Gynecological Cytology Tests:

- **Biomarker tests for p16 and Ki-67 can help triage abnormal cytology results:**
- P16 is of great significance for the screening of cervical cancer but by itself may not be sufficient for diagnosis.
- Ki-67 is a nuclear antigen that can be detected in the non-G0 phase of the cell cycle, marking the process of cell proliferation.



Images of Pap smears with applied dual p16/Ki67 immunocytochemical.

The future of gynecological cytology

- Artificial intelligence for enhanced accuracy in cell analysis.
- Liquid biopsy techniques for non-invasive cancer detection
- Advanced molecular diagnostics for personalized medicine.

These technologies promise earlier detection, improved patient outcomes, and more efficient, targeted treatment protocols.

Useful links

ThinPrep Pap Test Specimen Collection

https://youtu.be/hDzyhb_4Hu4

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