



IMPORTANCE OF PAPANICOLAOU STAINING IN GYNECOLOGIC CYTOLOGY

Agbo-inedu maria lhotu-owoicho¹, Ajit Pal Singh^{2*}

¹5th Semester Student, Bachelor in Medical Laboratory Technology (B.sc MLT), School of Allied Health Sciences, Sharda University, Greater Noida, UP

²Assistant Professor, Department of Medical Lab Technology, School of Allied Health Sciences, Sharda University, Greater Noida, U.P.

*Correspondence & Reprint Request: Ajit Pal Singh, Assistant Professor, Department of Medical Lab Technology, School of allied health sciences, Sharda University, Greater Noida, U.P.

ABSTRACT

As we all know pap staining also known as the universal smear is one of the most important procedures in cytology as it is used in gynecologic and non-gynecologic cytology smear. It is one of the most widely used stains in cytology, as it has reduced drastically the diagnosis of cervical cancer by 70% in highly developed countries. Pap smear is very important because it reveals the structure of the cell to be examined. examples include, the cytoplasm, nucleus and all cellular granules.

KEYWORDS: PAP Staining, Cytology, Importance, PAP smear, cervical

1. INTRODUCTION

A multicolored cytological staining procedure which was developed by **George Papanicolaou** in 1942[1]. This stain is said to have helped pathologist and laboratory researchers in making diagnosis worldwide. It is mainly noted for its viral detection of cervical cancer (3) formulations of this stain were published in 1942,1954 and 1960[2]. During the course of studies, it was noticed that there could be an improvement in our procedure for staining cervical and vaginal tissues [3]. It became a determinant for the diagnosis of premalignant and malignant lesions of the cervix. Malignant lesions on the cervix became the constant cause of death in women therefore making it one of the most common cancers in females.

The Papanicolaou[pap] test is a screening test done involving the uterine tissues and cells, it is considered to be simple, quick, painless and cost-effective [4].

2. USAGE

Pap staining is used to differentiate cells in smear preparation from various parts of the body, these specimens include;

- Sputum
- Synovial fluids
- Urine
- Cerebrospinal fluids
- Abdominal fluids
- Pleural fluids
- FNAC and other loose specimens containing smear

3. PRINCIPLE

The stain comprises of both basic and acidic dyes, while the basic dye stains the acidic cell components, the acidic dye stains the basic cell components. This occurs based on the ionic charges of the cell's constituents alongside the principle of attraction and repulsion of ions and dyes.



five different dyes are used in three solutions as the main reagents used in the stain they include:

- **Hematoxylin:** This natural dye stains the cell nuclear blue with its affinity for chromatin attaching itself to sulfate groups on the DNA molecule [5].
Harris' hematoxylin is known to be one of the most common dyes used cytological due to its efficiency [6]
- **Orange green 6:** known as the first acidic counterstain that stains the cytoplasm of matured and keratinized cells. The targeted structures are stained orange in distinct intensities [7]
- **EOSIN AZURE:** This is the second counterstain and it is a combination of **eosin Y**, **light green SF** and **Bismarck brown**. **EOSIN Y** stains the cytoplasm of matured squamous, nucleoli, RBC and cilia. The most commonly used staining solution in cytology are EA 31, EA 50, EA 65.
- **LIGHT GREEN SF:** Stains the cytoplasm of active cells like columnar cells, intermediate squamous cells and para basal squamous cells [8].
- **BISMARCK BROWN Y:** Oftentimes, it is disregarded because it stains nothing[9].

Composition and reagents of pap stain

- **Harris' hematoxylin:**
Hematoxylin = 5g
Ethanol = 50ml
Potassium Alum = 100g
Distilled Water(50) = 1000ml
Mercuric Oxide =225g
Glacial acetic acid = 20ml[10]
- **Orange green 6**
Orange Green (10% aqueous) = 50ml
Alcohol = 950ml
Phosphotungstic Acid = 0 – 15g
- **EA 50**
0.04 M light green SF = 10ml
0.3M eosin Y = 20ml
Phosphotungstic acid = 2g
Alcohol = 750ml
Methanol = 250ml
Glacial acetic acid = 20ml
N.B ensure to filter all stains before use.

4. PROCEDURE OF PAP STAIN(METHODOLOGY)

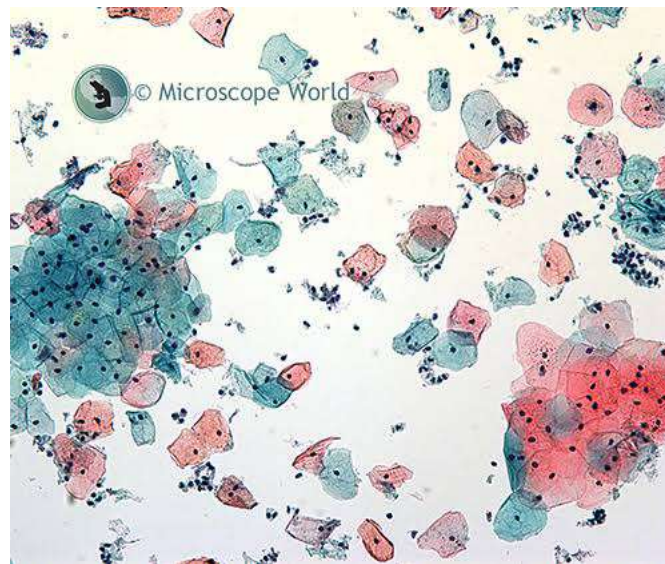
- Fix smear using 95% alcohol for 5-15 mins
- Rinse with tap water[hydration]
- Apply Harris hematoxylin dye for 1-3mins
- Rinse using tap water
- Dip in 95% alcohol [10 dips]
- Apply orange G-6 stain for 1.5 mins
- Dip in 95% alcohol [10dips]
- Apply eosin dye or other applicable dye for 2.5 mins
- Dip in 95% alcohol [10 dips] 2 changes
- Dehydrate in absolute alcohol[methanol] 1min
- Clear with xylene 1min[11]



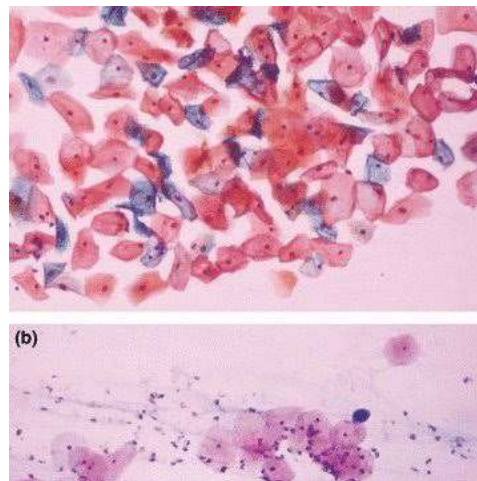
5. RESULTS

The dyes would stain different cell components with varying intensities and color

- Nuclei: blue
- Cytoplasm: different shades of red, pink, yellow green-gray
- Acidophilic cells: pink
- Eosinophils: orange or red
- Basophilic cell: green to blue-green
- Rbc's: orange to dark pink
- Superficial cells: pink
- Parabasal or intermediate cell: green
- Candida[fungi]: red[12]



Pap smear test for cervical cancer seen under a microscope.



Normal cervical showing cytoplasmic green staining of intermediate cells and orange staining of superficial cells [13]



6. CONCLUSION

Times are changing making it easy to diagnose cervical cancer at its early stage with the help of pap staining, thereby reducing the morbidity and mortality rate of women with cervical cancer. Awareness should be created on the important diagnostic procedure to help save lives.

7. REFERENCES

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