

## MODIFIED RAPID PAPANICOLAOU STAIN AS A SUITABLE ALTERNATIVE TO STANDARD PAPANICOLAOU STAIN

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### ABSTRACT

**Objective:** Papanicolaou stain is a multichromatic stain used to differentiate cells in smear preparations of various bodily secretions. However, it is not fully standardized technique because it comes in several versions. This study was done to design a method that requires hardly any alcohol during staining of cytology and thereby to reduce the expense and to search for staining of cytology smear that is qualitatively as good as standard Papanicolaou stain for cytological examination and for cervical cancer screening. **Methods:** A number of smears were taken from patients attending the Cytopathology department of central public health Laboratory in Baghdad. Two smears from each patient were collected and stained by standard

Papanicolaou stain and the modified method of stain separately. All the modified and standard PAP stained smears were screened by senior pathologists and compared using various parameters like the quality of cytoplasmic stain and its transparency and nuclear details like margin, nucleolus and chromatin pattern. **Results:** The total number of patients was 76, and for each patient two smears were taken. One slide was stained using conventional technique and the other was stained using the modified one. The staining quality of the modified technique was approximately similar to conventional PAP stain. **Conclusion:** Modified Papanicolaou stain, in comparison to conventional Papanicolaou, provides a suitable, excellent & rapid alternative for cytological screening with minimum cost.

**KEYWORDS:** Papanicolaou stain, cytology, cervical cancer.

### INTRODUCTION

Papanicolaou stain (also Papanicolaou's stain and Pap stain) is a multichromatic staining histological technique developed by George Papanikolaou, the father of cytopathology.<sup>[1]</sup>

It is the universal stain for cytological examination. It yields a polychromatic, transparent staining reaction with clear nuclear and cytoplasmic features.<sup>[1,2]</sup>

Pap staining is used to differentiate cells in smear preparations of various bodily secretions; the specimens can be gynecological papanicolaou smears (Pap smears), sputum, brushings, washings, urine, cerebrospinal fluid, abdominal fluid, pleural fluid, synovial fluid, seminal fluid, fine needle aspiration material, tumor touch samples, or other materials containing cells.<sup>[1]</sup>

Pap staining is a very reliable technique. As such, it is used for cervical cancer screening in gynecology. The entire procedure is known as Pap smear.

The classic form of Pap stain involves five dyes in three solutions.<sup>[3]</sup>

- A nuclear stain, hematoxylin, is used to stain cell nuclei.
- First OG-6 counterstain (-6 denotes the used concentration of phosphotungstic acid; other variants are OG-5 and OG-8). Orange G is used. It stains keratin. Its original role was to stain the small cells of keratinizing squamous cell carcinoma present in sputum.
- Second EA (Eosin Azure) counterstain, comprising of three dyes; the number denotes the proportion of the dyes, eg. EA-36, EA-50, EA-65.
  - Eosin Y stains the superficial epithelial squamous cells, nucleoli, cilia, and red blood cells.
  - Light Green SF yellowish stains the cytoplasm of all other cells.
  - Bismarck brown Y.

Pap stain is not fully standardized; it comes in several versions, subtly differing in the exact dyes used, their ratios, and timing of the process.<sup>[2]</sup>

The descriptions of the compositions of the staining solutions vary by source and differ even in Papanicolaou's own publications. Mixtures of the same name from different vendors therefore can differ in composition, occasionally producing different or poor results.

Most laboratories use commercial stains and each will consider their modification of the original technique to be the optimum. However, it utilizes a considerable amount of ethyl alcohol and takes a considerable time.

Therefore, there is a continuous search of a standardized rapid method suitable for cytological examination and for cervical cancer screening.<sup>[2]</sup>

This study was done to design a method that requires hardly any alcohol during staining of cytology and thereby to reduce the expense and to search for staining of cytology smear that is qualitatively as good as standard Papanicolaou stain for cytological examination and for cervical cancer screening.

## **MATERIALS AND METHODS**

A number of smears were taken from patients attending the Cytopathology department of central public health Lab.

Two smears from each patient were collected and stained by standard Papanicolaou stain and the modified method of stain separately.

Only in the first step for fixation and in last step for dehydration, absolute alcohol was used which is same as standard Papanicolaou stain.

### **Methods for modified Papanicolaou stain**

- Removing slides from the fixative (95% Ethyl Alcohol)
- 1% acetic acid 10 dips
- Tap water 10 dips
- Harris's Hematoxylin (1 minute)
- Tap water 10 dips
- 1% acetic acid 10 dips
- OG-6 (1 minute)
- 1% acetic acid 10 dips
- EA-65 (1 minute)
- 1% acetic acid 10 dips
- Absolute Ethyl Alcohol 10 dips
- Equal parts of Absolute Ethyl Alcohol & Xylene 10 dips
- Xylene 10 dips
- Mount by Canada balsam

Total time for staining in the present method was about 6 minutes (Conventional PAP stain takes about 20 minutes). In Papanicolaou stain, fixed smears are passed through a series of descending grade of ethyl alcohol before nuclear staining. These ethyl alcohol grades were replaced by single 1% Acetic acid step. Harris's hematoxylin is used in both methods for nuclear staining.

Before staining with Orange G 6 the two changes of dehydrating Ethyl alcohol grades were replaced by 1% Acetic acid in the modified method.

The cytoplasmic stains (OG6 and EA65) were the same in both methods.

Two changes of 95% ethyl alcohol with Standard PAP stain after OG6 were replaced by 1% Acetic acid (10 dips).

In standard PAP stain, final dehydration is done by two changes of absolute alcohol. In the modified technique, the smears were washed in 1% Acetic acid & final dehydration by absolute ethyl alcohol 10 dips.

With PAP stain clearing is done by one change of alcohol-xylene followed by 2 changes of xylene (10 dips each).

In the modified staining technique, clearing is done by one change of alcohol-xylene followed by single change of xylene (10 dips each).

All the modified and standard PAP stained smears were screened by senior pathologists of different units of our Lab. and screened separately without any comparison and bias.

Modified smears were compared with conventional PAP smears using various parameters like the quality of cytoplasmic stain and its transparency and nuclear details like margin, nucleolus and chromatin pattern.

An absolute confidentiality of the patients' vital information was maintained for ethical purposes.

## RESULTS

The total number of patients was 76, and for each patient two smears were taken using traditional Ayre's spatula.

One slide was stained using conventional technique and the other was stained using the modified one.

The staining quality of the modified technique was approximately similar to conventional PAP stain.

Figure.1 compares the staining quality of the modified & conventional Papanicolaou stain.

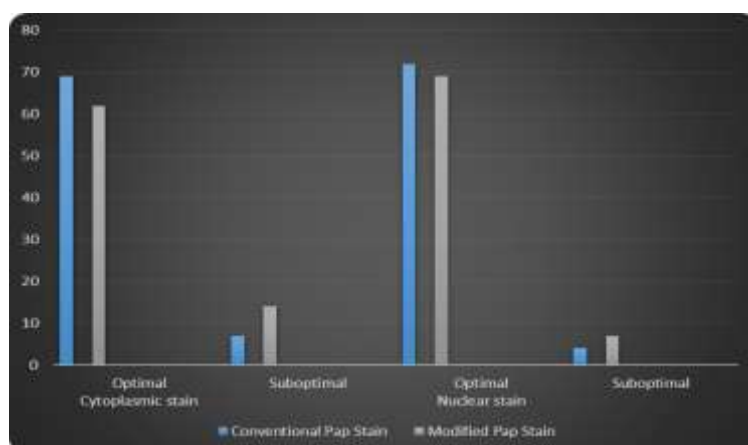
Sixty-two smears out of 76 cases showed good transparency of cytoplasm. The rest were suboptimal due to thick smears. Nuclear details like margin, nucleolus and chromatin pattern were optimum in most of the cases, 69 out of 76.

Staining reaction of non-epithelial cells like RBC, WBC, bacteria were well preserved as found in standard papanicolaou stain (Figure.2).

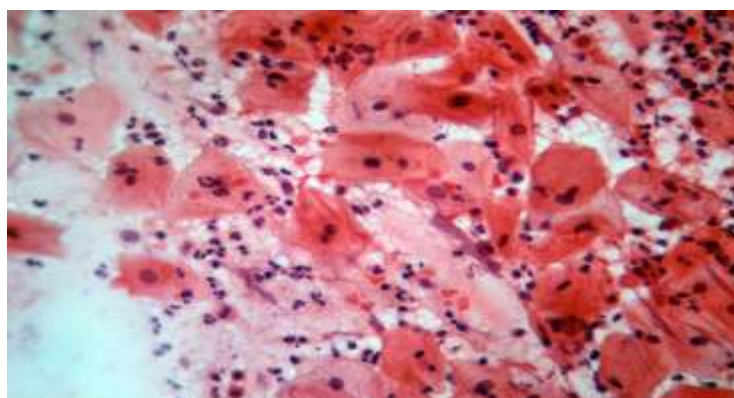
The staining quality remained well preserved for 6 months without any fading.

The cytoplasmic stain was optimal in 62 smears out of 76 (82%). The cyanophilia & eosinophilia of the cells (Figure.2) were comparable with Papanicolaou stain. The rest 14 smears showed suboptimal stain because the stain could not penetrate the thick part.

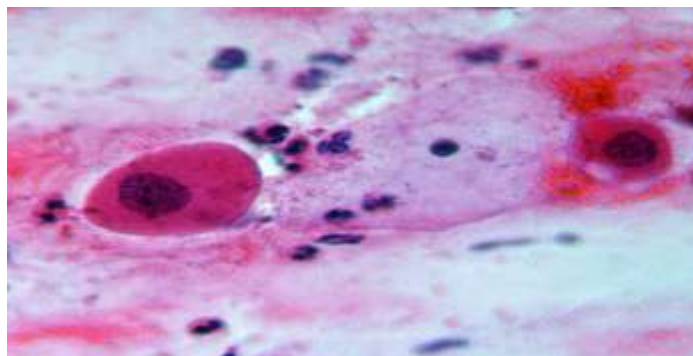
In 90% (69 out of 76 smears) cases, nuclear staining (Figures.3 & 4) showed optimal features. Only in 10% occasions, the nuclear staining was pale & suboptimal. The staining quality was preserved for 6 months.



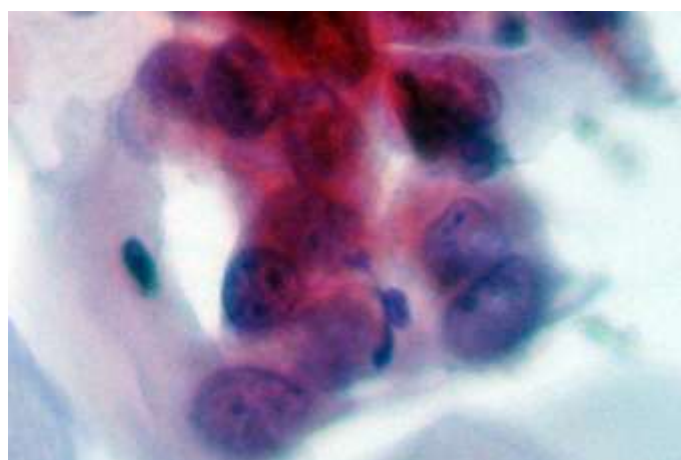
**Fig-1: Results of conventional Papanicolaou and modified methods**



**Fig. 2: smear stained by modified technique showing mature Superficial cell, intermediate cell with inflammatory cells in the background.(MP)**



**Fig. 3:** smear stained by modified technique showing two moderate dysplastic cells and a mature superficial cell (HP)



**Fig-4:** smear stained by modified technique showing clumps of severe dysplastic cells(HP)

## DISCUSSION

The cervico-vaginal cytology is universally known as PAP test as it was popularized by George Papanicolaou. Today it is widely used both as a screening test in asymptomatic population & in the follow –up patients with cervical carcinoma.

Following mass screening there has been reduction of 38% to 57% in the overall incidence of invasive carcinoma and a reduction of 67% in the incidence of clinically evident carcinoma. The original Papanicolaou stain of PAP smears had undergone various modifications.<sup>[1,3,4]</sup> in different laboratories .Certain techniques.<sup>[2]</sup> gives cytomorphological pictures as good as Papanicolaou stain but it requires a large quantity of expensive ethyl alcohol. In this modified technique, ethyl alcohol is replaced in most of the steps by 1% Acetic acid. Acetic acid will act as mild dehydrating agent. It is cheap and easily available.<sup>[5]</sup> In this study, the cytoplasmic stain was optimal in 62 smears out of 76 (82%).The cyanophilia & eosinophilia of the cells

(Figure.2) were comparable with Papanicolaou stain. The rest 14 smears showed suboptimal stain because the stain could not penetrate the thick part. Nuclear staining requires demonstration of sharp nuclear features with crisp chromatin for diagnostic accuracy. This study showed, in 90% (69 out of 76 smears) cases nuclear staining (Figures.3 & 4) showed optimal features. Only in 10% occasions, the nuclear staining was pale & suboptimal. The staining quality is preserved for 6 months.

The cost factor plays the pivotal role in any organized screening program, especially in developing country with high prevalence of the disease concerned. The modified technique costs about 25% of total cost of standard Papanicolaou stain<sup>[5]</sup>; thereby it is cost-effective in mass screening program. The method is also relatively rapid & suitable for the screening test.

### CONCLUSION

Modified Papanicolaou stain, in comparison to conventional Papanicolaou, provides a suitable, excellent & rapid alternative for cytological screening with minimum cost.

Overall, the use of absolute alcohol is minimum; it is used only during fixation and for dehydration before mounting. The stain preservation is also good in this method. It may be considered the suitable alternative to standard Papanicolaou stain for cervical cancer screening programs.

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