

# Comparison of Conventional Papanicolaou Stain with Enviro Papanicolaou Stain in Buccal Smear

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**Abstract:** *The traditional screening method for cervical cancer is the PAP smear. As practiced conventionally PAP stain is expensive utilizing a considerable amount of alcohol and consumes a lot of time. This study is to find out a standard technique which is superior or comparable to conventional PAP staining, rapid and cost effective with minimal alcohol use. This study aimed to compare Conventional Papanicolaou stain and Enviro PAP stain in buccal smear. We collect two buccal smears from 500 persons and the smears were stained with PAP and Enviro PAP. Then examine the slides under microscope to assess its utility depending on quality of staining assessed by nuclear characteristics, cytoplasmic details, cell morphology, artifact and background. The Enviro PAP staining quality is closely related to Conventional PAP staining Quality. Enviro PAP procedure has minimum alcohol use and technician friendly protocol that does not compromise on staining quality and diagnostic standards. This staining procedure is simple and environmental friendly. Using Enviro PAP can substantially reduce a laboratory's annual staining reagent purchase and Xylene disposal cost. It can be easily adopted as a suitable alternative to the expensive and time consuming standard protocol for mass screening of cervical cancer in limited resource settings.*

**Keywords:** Papanicolaou Stain (PAP), Ultra Fast Papanicolaou Stain (UFP), Modified Ultrafast Papanicolaou Stain (MUFP), Eosin Azure (EA), Orange GELB (OG).

## 1. Introduction

Since 1947, there have been numerous improvements to the PAP stain and its staining method, including Rapid PAP staining, Ultrafast PAP staining (UFP), Modified Ultrafast PAP staining (MUFP) Enviro PAP staining and cervical acid phosphatase PAP stain. Since different stain formulations are sold by various vendors and cytotechnologists are trained in varied techniques, each lab should have its own staining protocols, which should be standardized.

The staining process requires ethanol for processing, which is frequently expensive and challenging to obtain. Several alcohol substitutes are employed. Alcohol has environmental risks. Thus, it is necessary to standardize a Papanicolaou staining procedure that is quick, cheap, and uses environmental friendly reagents. The major goal of this study is to identify a standard approach that is better than or equal with traditional PAP staining, quick, economical, and with minimum alcohol consumption minimized [1].

We are collecting buccal smears, because it can be obtained from non-invasive collecting methods. The buccal mucosa composed of squamous epithelial cells. Its collection method is a painless method. It is quick, low-cost collection compared to tissues such as blood. It collected by swabs or brushes. Buccal cells are easily available.

In this study, we compare buccal smear PAP staining with environmental PAP staining. The results of the environmental friendly Enviro PAP stain were comparable to those of the traditional PAP method. With Enviro PAP staining, tap water is used in place of the alcohol-based hydration and dehydration baths. Our goal was to look for quick staining techniques that didn't sacrifice the quality of the cell morphology for cost or speed.

## 2. Literature Review

Pap stain was developed by Greek doctor George Nicholas Papanicolaou to know the variation in cellular maturity and metabolic activity in vaginal smears. The original papstain was published in 1942 and later it was modified. Since the introduction of papstain by G. N. Papanicolaou it has undergone various modifications [2, 3].

A study conducted by Gary. W. Gill and his colleagues on the topic "Enviro-Pap: An Environmental friendly, economical, and effective pap stain". In this study the number of samples were not mentioned. In their study they find that the Enviro PAP is superior. Enviro PAP reduces the use of ethanol, bluing agents and xylene. They also find that the Enviro PAP reduces cost [4].

Study conducted by Sai Sudha. M and colleagues on the topic "Papanicolaou stain - Review" they compare conventional PAP stain with rapid PAP stain, ultra-fast PAPstain, modified ultrafast PAP stain, Enviro-PAP stain and rapid economic acetic acid PAP stain. They found that Enviro PAP stain uses water instead of 95% ethanol to remove carbowax from spray fixed slides, plain tap water for bluing and xylene uses along with water scavenging beads. It doesn't use graded alcohols. Therefore, Enviro PAP is eliminating all alcohol baths, bluing agents and xylene disposal. It can also be easily implemented in other cytology labs and even can be extend to histopathology lab. It decreases the lab annual staining reagent purchase. The only disadvantage of Enviro PAP is over staining in OG, which can be easily prevented by carefully limiting the staining time [5].

R. N. Gachie et al. conducted a study on "A comparison of modified and standard Papanicolaou staining methods in the assessment of cervical smears. Their sample size is 162

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women and was determined statistically. A Pap smear was then taken from the cervix using a cervix broom and was dropped into a vial containing 10-15 ml of Pap spin collection fluid. The liquid-based method was used to prepare the smear. Out of 162 paired smears examined in the study, 159 pairs were found satisfactory for evaluation. Three pairs of smears were excluded as they were unsatisfactory for evaluation due to scanty cellularity and a repeat was recommended. The staining quality was compared in both methods [6, 7].

### 3. Methodology

The buccal smear collection procedure was performed in our laboratory by each person's itself by the standard method. The buccal smear is collected by gently scraping the inside of the cheek with a clean slide or toothpick then spreading evenly on a clean grease free slide. A total of minimum 2 smears were made from each person. One smear is submitted for conventional Papanicolaou (PAP) stain and the other one is for Enviro PAP stain.

#### Standard Pap Staining Procedure

Reagent	Time
80% Ethyl alcohol	10 dips
70% Ethyl alcohol	10 dips
50% Ethyl alcohol	10 dips
Distilled water	2 Rinse
Harris haematoxylin	6 minutes
Distilled water	2 Rinse
95% Ethyl alcohol	10 dips
70% Ethyl alcohol	10 seconds
Distilled water	10 dips
Lithium carbonate	1 dip
Running tap water	10 dips
0.5% aqueous HCL	2 dips
OG6	2 minutes
95% Ethyl alcohol	10 dips
95% Ethyl alcohol	10 dips
EA36	10 dips
95% Ethyl alcohol	10 dips
95% Ethyl alcohol	10 dips
100% Ethyl alcohol	10 dips
Xylene	10 dips
Xylene	10 dips
Mount in DPX	

#### Enviro PAP Staining Procedure

Reagent	Time
Tap water	10 dips
Tap water	10 dips
Harris haematoxylin	1-2 minutes
Tap water	10 dips
Tap water	10 dips
Tap water	10 dips
Tap water	10 dips
OG6	10 seconds
0.5% acetic acid	10 dips
0.5% acetic acid	10 dips
0.5% acetic acid	10 dips
EA36	2-3 minutes
0.5% acetic acid	10 dips
0.5% acetic acid	10 dips
0.5% acetic acid	10 dips
Absolute ethanol	10 dips

Absolute ethanol	10 dips
Absolute ethanol	10 dips
Xylene	10 dips
Xylene	10 dips
Mount in DPX	

We are adding 100% Isopropyl alcohol instead of Absolute ethanol and the life of xylene is increased by adding silica gel in xylene bath.

#### Evaluation of Staining Quality

The stained smears were examined and compared for quality of staining method employed in terms of nuclear staining, cytoplasmic staining, artifacts and background of the smear. Each criteria was graded and scored individually using the grading criteria as shown below.

NUCLEAR STAINING	
No cells seen	0
Poor staining	1
Good nuclear staining	2
Excellent nuclear staining	3
CYTOPLASMIC STAINING	
No cells seen	0
Poor	1
Moderate	2
Excellent	3
BACKGROUND	
No cells seen	0
Dirty background	1
Obscured background	2
Clean background	3
ARTIFACTS/STAIN DEPOSITS	
No cells seen	0
Drying artifacts / stain deposits	1
Some artifacts / stain deposits	2
No artifacts / stain deposits	3

### 4. Result and Discussion

In this study we evaluated the cytomorphological traits of buccal smears stained with conventional PAP stain and those stained with Enviro PAP with Isopropylalcohol Fixation. Enviro PAP can significantly lower a laboratory's annual cost for Xylene disposal and staining reagent purchases. Enviro PAP is equal to conventional PAP stain by quality of staining in all aspects so it can be implemented in any cytology laboratory.

### 5. Conclusion

Enviro PAP procedure has minimum alcohol use and technician friendly protocol that does not compromise on staining quality and diagnostic standards. This staining procedure is simple and environmental friendly. Using Enviro PAP can substantially reduce a laboratory's annual staining reagent purchase and Xylene disposal cost. It can be easily adopted as a suitable alternative to the expensive and time consuming standard protocol for mass screening of cervical cancer in limited resource settings.

## 6. Future Scope

The current scientific staining techniques majorly involve the usage of non-ecofriendly reagents like xylene and alcohol. This research pinpoints the ecofriendly and effective usage of EviroPAP as a replacement of xylene and alcohol in the modern staining techniques. So in order to promote the usage of ecofriendly products in the laboratories, it's very much advisable to start practicing the usage of EviroPAP.

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