**International Journal of Current Advanced Research**
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 7; Issue 5(F); May 2018; Page No. 12595-12598
DOI: http://dx.doi.org/10.24327/ijcar.2018.12598.2219

**Research Article**

# MODIFIED ULTRA FAST PAP STAIN IN CYTOLOGY IN COMPARISION WITH REGULAR PAP STAIN AND MGG

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**A R T I C L E I N F O**

***Article History:***

Received 16th February, 2018
Received in revised form 14th March, 2018 Accepted 8th April, 2018
Published online 28th May, 2018

***Key words:***

PAP, MGG, MUFP

**A B S T R A C T**

**Background**: Quick diagnosis of Fine needle aspiration cytology (FNAC) plays an important role in efficient medical practice. Ultrafast Papanicolaou stain was introduced, which is a hybrid of Romanowsky and PAP stain. This method reduce reduces the staining time to 90 seconds because of the use of fixative like alcoholic formalin andalso enhances staining the quality.

**Aims and objectives**: To assess the quality of MUFP stain and to find the advantages over routine stains used in cytology.

**Materials and methods:** This study was included100 cases of FNA from thyroid, breast, and lymph node lesions. Minimum 3 smears made and stained with routine Pap, MGG and MUFP stain. All smears were compared in 6 parameters and Quality index is calculated.

**Results:** MUFP is a excellent staining method for studying FNA material from all three organs like thyroid breast and lymph node lesions.

**Conclusion:** MUFP staining is quick, reliable and can be done with easily available reagents and is very useful in countries like India.



## INTRODUCTION

The papanicolaou staining technique is a polychromatic staining method elaborated by George N Papanicolaou, who is considered to be the father of cytology.[1] Pap stain is used to differentiate the cells in smear preparations of various body fluids, gynecological smears and fine needle aspiration material from various organs.There has been a lot of controversy between wet-fixed smears (H&E,PAPstain) and air-dried smears stained with Romanowsky's stain.[2] In fact, both are complementary, but H&E and Pap staining permit better assessment of nuclear features and are preferred by many histopathologists.[3] Quick diagnosis of FNAC plays an important role in efficient medical practice.To overcome this, ultrafast papanicolaou stain was introduced by yang and Alvarez in 1994 which is a hybrid of romanowsky stain and pap stain.[4] It not only reduces the time for pap stain to 90 seconds, but also enhances the quality.[5]

## MATERIALS AND METHODS

This prospective study was carried out in the Department of pathology, Coimbatore medical college, for the period of one year. The Study includes fine needle aspiration from thelesions of thyroid, breast, and lymph nodes.

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Fine needle aspiration from other organs are excluded. Total number of cases studied – 100. Thyroid – 40, Breast –40, Lymph node – 20. The Fine needle aspiration was done in our hospital by standard technique. A minimum 3 smears were obtained on glass slides. Out of which 1 smear was fixed in 95% ethanol for a minimum of 15 minutes. These smears were stained for pap. The remaining 2 smears were air dried out of which one was stained by MGG stain and other smear was rehydrated with normal saline and subsequently fixed in alcoholic formalin and stained by MUFP stain. The Statistical analysis, the data are reported as mean +/-SD or median and frequencies are expressed in percentages. Anova used for quantitative variables. The chi square test & Fisher exact test used for variables between groups. A p value of <0.05 is significant using SPSS, version 16.0 for windows.

***Scoring System Used In Assessment of Staining***

| Parameter | SCORE=1 | SCORE=2 | SCORE=3 |
|---|---|---|---|
| Background | Hemorrhage | Clean | |
| Overall staining | Poor | Average | Good |
| Cell morphology | Poorly Preserved | Moderately Preserved | Well Preserved |
| Nuclear Characteristics | Smudgy Chromatin | Moderately Crisp Chromatin | Crisp Chromatin |
| Cytoplasmic details | Unsatisfactory | Suboptimal | Optimal |
| Air drying artifacts | >50% | <50% | 0% |

The maximum score was 17 for a single case, it was consider into account of all the six parameters, The "Quality Index" was calculated as the ratio of actual score obtained to the maximum score possible. Quality Index= actual score obtained /maximum score (17)

Quality Index for each of the three stains of the three organs was compared.

## OBSERVATION AND RESULTS

**Table No 1**

| Age Distribution | | | | |
|---|---|---|---|---|
| | Cases | | | |
| Age | Thyroid | Breast | Lymph node | Total |
| <20 | 5 | 6 | 0 | 11 |
| 21-30 | 8 | 6 | 2 | 16 |
| 31-40 | 13 | 7 | 4 | 24 |
| 41-50 | 9 | 12 | 5 | 26 |
| 51-60 | 3 | 6 | 3 | 12 |
| 61-70 | 2 | 2 | 4 | 8 |
| >70 | 0 | 1 | 2 | 3 |
| Total | 40 | 40 | 20 | 100 |

**Table No 2** Association of overall staining with Study cases

| | Cases | | |
|---|---|---|---|
| **Overall staining** | **PAP** | **MGG** | **MUFP** |
| • Poor | 0 | 3 | 0 |
| • Average | 39 | 88 | 16 |
| • Good | 61 | 9 | 84 |
| Total | 100 | 100 | 100 |

**Association of cell morphology with study cases**

| | Cases | | |
|---|---|---|---|
| **Cell morphology** | **PAP** | **MGG** | **MUFP** |
| • Poorly preserved | 0 | 5 | 0 |
| • Moderately Preserved | 31 | 85 | 14 |
| • Well preserved | 69 | 10 | 86 |
| Total | 100 | 100 | 100 |

## DISCUSSION

Fine needle aspiration cytology (FNAC) is one of the least expensive (most economical), quickest and simplest methods available for early diagnosis of various palpable and deep seated lesions. Since its establisment, PAP stain remains the traditional and most widely used stain, not only for the gynecological cytology, but also for various lesions of other organ.[6] The different staining methods of air dried smears are MGG, Jenner-Giemsa and Diff-Quickstain .But they will not offer the transparency in the study of subtle nuclear features as seen by the PAP stain.[7]

To overcome these problems, ultra-Fast pap (UFP) stain was developed by Yang and Alvarez. It is a hybrid of pap and Romanowsky stains. The staining time is 90 seconds.[1,8] Further modification of UFPstain (Modified ultra-Fast pap stain) done by Kamal *et al* from India, to overcome the shortage of Richard-Allan hematoxylin and Richard-Allan cytostain in Indian set-up. Staining time of this method is 130 seconds and with well appreciated cytomorphology.[5,9]

In this study cytomorphology of modified ultrafast papanicolaostain (MUFP) was compared with conventional papanicolaou stain and May Grunwald Giemsa stain. MUFP stain-rehydrated air dried smear. PAP stain-ethyl alcohol fixed wet smear. MGG stain-air dried smear. All 3 stains quality was evaluated in six parameters, as background, Overall staining, cell morphology, nuclear characteristics, cytoplasmic features and air-drying artifacts.

The quality index of all three stains were calculated and compared with various organs like thyroid, Breast and lymph node.Ultrafast pap stain was done in thyroid, breast and lymph node lesion by Shinde *et al*.[6] Quality indices of our study were compared with quality indices ofShinde's study showed similar results. Quality index of MUFP stainin the same three organs are also compared with rapid pap stain by Priyanka Choudhary *et al* and showed similar results.[6,13] background mufp stain lyses blood cells , making the smear much thinner and clearer. RBC free background provide better cytomorphologic feactures. Rehydration solution of MUFP is normal saline. Rehydration solution of MUFP is normal saline. This provide transparent air dried cells and well preserved nuclear details.[1,3] Cells were appear larger due to air drying with red stained distinct nucleoli. Our study proves that air-dried smears rehydrated with normal saline providesclean background as compared to wet fixed and air dried smears.

***Overall Staining***

84% of MUFP stain showed good overall staining followed by PAP(61%), and MGG (9%). 88% of MGG stain showed average overall staining.

***Cell Morphology***

Well preserved cell morphology is maximum for MUFP (86%) followed by Pap (69%) and MGG stain (10%).

***Nuclear Characteristics***

89% of Pap stain showing crisp nuclear chromatin followed by MUFP stain (69%) and MGG stain (9%). 78% of MGG showing moderately crisp nuclear chromatin.

***Cytoplasmic Details***

Grading of cytoplasmic features are unsatisfactory, suboptimal and optimal. Optimal cytoplasmic features are seen in 92% of MGG stained smears followed by MUFP (82%) and Pap (59%) stain.

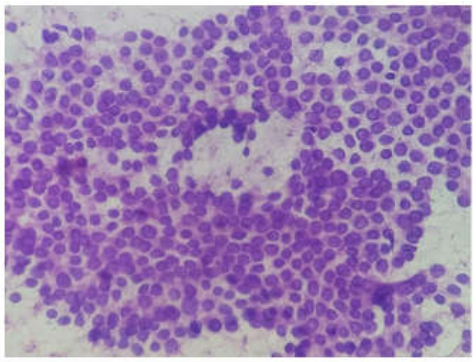
***Air-Drying Artifacts***

No air drying artifacts are seen in 86% of MGG, 82% of MUFP and followed by 44% of Pap stain. Kamal *et al*. found the problem of wet fixation, but the air drying artifacts can be eliminated by rehydration of air dried smears as in MUFP.[6] Thus our study proved that air drying technique and rehydration of air dried smears was associated with less air drying artifacts as compared to wet fixation. Our study compares the Modified Ultrafast Papanicolaou Stain (MUFP) with conventional Papanicolaoustain and MGG stain. MUFP is quick and has the advantage that Thebackground is clean and shows very less air drying artifact.

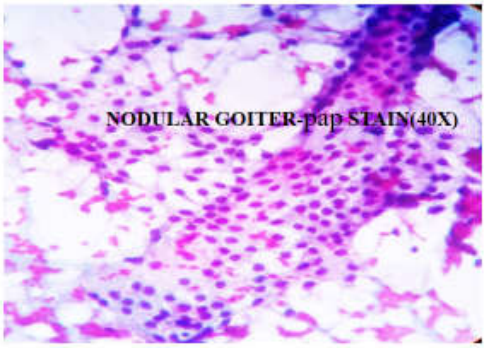
## CONCLUSION

Modified ultrafast pap stain is a excellent staining method for studying FNA material from all three organs like thyroid, breast and lymph node lesions. MUFP stain showed maximum score for all four parameters and shows optimal cytoplasmic features in 82% of cases and crisp nuclear chromatin in 69% cases. Fixative of MUFP stain is alcoholic formalin. It provide lesser staining time for fixation and makesnucleoli to appear red and prominent, Compared to conventional Pap stain.

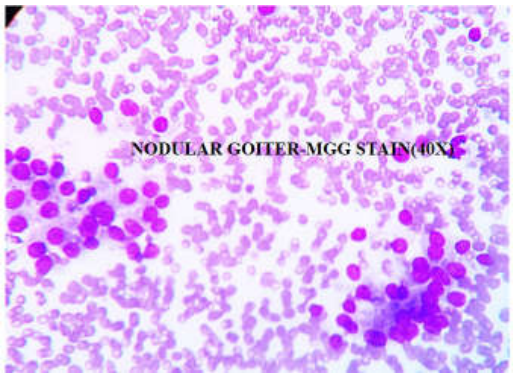
Lesser time for staining along with excellent morphologic quality is the need of the hour in any cytology laboratory. MUFP very easily fulfils these parameters either equal to or even better than pap Technique for cytologic staining and organ study. MUFP staining is quick, reliable and can be done with easily available reagents and is very useful especially in countries like India.



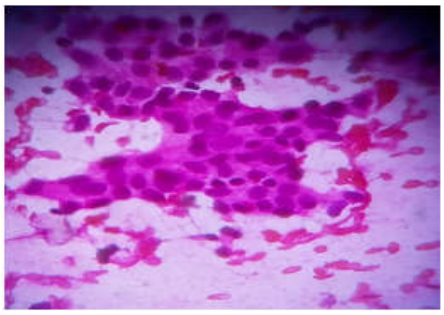
**Nodular Goiter – Mufp Stain (40X)**



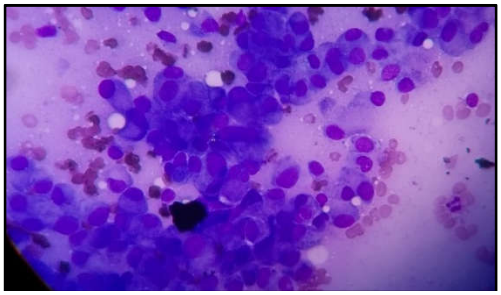

**Nodular Goitre – Pap Stain (40X)**



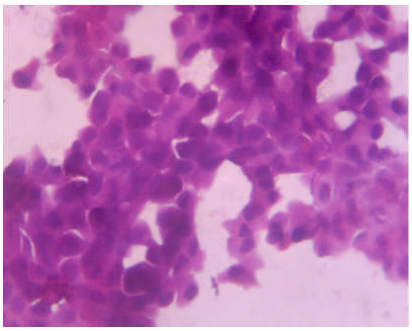

**Nodular Goiter –MGG Stain (40X)**



**Ductal Carcinoma – Pap Stain (40X)**

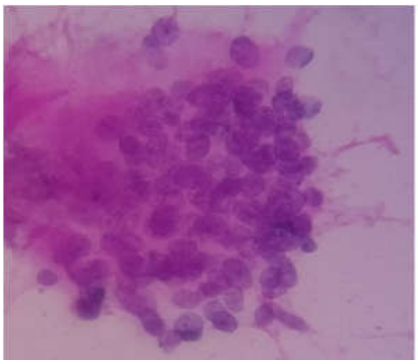


**Ductal Carcinoma – MGG (40X)**

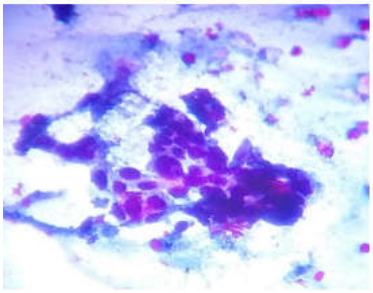


**Ductal Carcinoma – MUFP Stain (40X)**

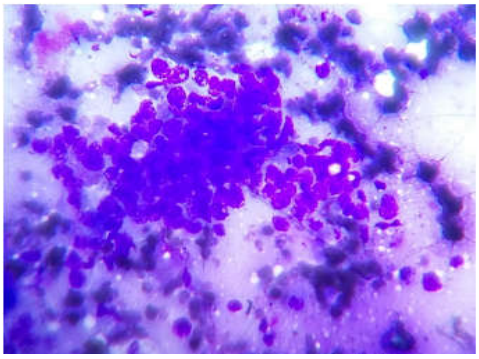
LYMPH NODE-METASTATIC CARCINOMATOUS DEPOSITS MUFP STAIN(40X)



LYMPH NODE-METASTATIC CARCINOMATOUS DEPOSITS PAP STAIN(40X)



**SQUAMOUS CELL CARCINOMA SECONDARIES IN LYMPH NODE, MGG stain, 40x**



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**How to cite this article:**

Selvi Palanisamy and Praba Victor (2018) 'Modified Ultra Fast Pap Stain in Cytology in Comparision with Regular Pap Stain and Mgg', *International Journal of Current Advanced Research,* 07(5), pp. 12595-12598.
DOI: http://dx.doi.org/10.24327/ijcar.2018.12598.2219

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