



RESULTS OF PROSAFE PAP IN CERVICAL CYTOLOGY

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ABSTRACT Papanicolaou staining is recommended for routine cervical cytology and cervical cancer screening. Our study was conducted by adapting modified staining technique which involves replacement of use of descending grades of alcohol with one step of 80% isopropyl alcohol. Prosafe papanicolaou staining was intended to reduce the staining time and cost of staining without compromising the quality or cytodiagnosis of the smear.

KEYWORDS : Conventional Pap, Prosafe Pap, Cervical cytology.

INTRODUCTION

Papanicolaou staining technique is a polychromatic staining method elaborated by George N Papanicolaou who is considered to be father of cytology .Pap stain is a universal stain used for gynecologic and non-gynecologic cytology smear. Pap test has decreased incidence of cervical cancer by 70% in developed countries.The principle of pap stain is to clearly distinguish between basophilic and acidophilic cell components and obtain detailed chromatin pattern.The conventional pap staining of cervical smears has undergone modifications to decrease turn around time. In this study,we compared overall staining, background, cytoplasmic and nuclear features of cells with conventional pap stain.

AIM

To assess efficacy of prosafe pap stain as an alternative method to conventional pap stain .

MATERIALS AND METHODS

200 cervical pap smears collected from unfixed total hysterectomy specimens received in S Nijalingappa medical college. Two slides prepared from each specimen and the smears were immediately fixed in 80% isopropyl alcohol. One set of smears was stained by standard conventional pap staining procedure followed in our laboratory .The other set was stained by prosafe pap stain.The coded smears were examined by cytopathologists .The nuclear and cytoplasmic parameters for staining quality and morphological details were assessed.The smears were individually compared and analysed for staining quality and morphology.

Steps for staining:

Table 1:

Conventional method	Prosafe Pap
95% Alcohol (for fixation)	80% Isopropyl alcohol (For fixation)
Tap water 10 dips	Hydrate smear with tap water - 30 secs
Tap water 10 dips	-
Harris hematoxylin 2 minutes	Harris hematoxylin - 30 secs
Tap water 10 dips	-
Tap water 10 dips	-
Scott's solution 2 minutes	Wash in alkaline tap water by adding Liquor ammonia - 30 secs
Tap water 10 dips	-
Tap water 10 dips	-
95% alcohol 10 dips	Dehydrate smear with 80% IPA - 30 secs

95% alcohol 10 dips	-
OG 6 1 minute	Stain with equal parts of OG-6 and EA -36 30 secs
95% alcohol 10 dips	Wash in isopropyl alcohol -30 secs
95% alcohol 10 dips	-
95% alcohol 10 dips	-
EA 10 minutes	-
95% Alcohol 40 dips	-
95% alcohol 40 dips	-
95% Alcohol 20 dips	-
100% Alcohol 10 dips	-
100% Alcohol 10 dips	-
100% Alcohol 10 dips	-
Xylene 10 dips	-
Xylene 10 dips	-
Xylene 10 dips	-
DPX mount coverslip	DPX mount coverslip

The total staining time was 5 minutes. The quality of prosafe pap staining was assessed by background, cell morphology, nuclear characteristics of the cells.

Scoring sytem used in assessment of staining:

Table 2:

Parameter	Score =1	Score=2	Score=3
Background	Hemorrhage	Clear	
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
Airdrying Artefacts	>50%	<50%	0%

The maximum score was 17 for a single case, it was considered into account all 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).

RESULTS

Total 100 cases were included in the study. Majority of the women were in the age group between 40 to 50 years .On examination of smears , majority of the cases showed no significant pathology, few

had chronic cervicitis and cervical carcinoma. Frank malignancy was detected in 08 cases. Cytological study of the cervical smears were done and assessed using parameters given in table.

In prosafe pap stained smears , nuclear chromatin appears sharp and crisp and nuclear membranes were also well demarcated. Excellent nuclear and cytoplasmic features were seen in 90% cases .This was followed by score of 17 in 90% of cases with optimal cytomorphology whereas a score of 10 was seen in 8% cases with suboptimal cytomorphology in conventional pap. There were no cases of poor preservation in any of the prosafe pap smears. Excellent nuclear features were seen in 72% cases and optimal cytoplasmic details were observed in 86% cases by conventional pap staining method.The conventional pap stain gave the usual cytoplasmic pink-red , blue green and orange brown variations and keratin stained orange. The prosafe pap maintained cytoplasmic transparency and revealed striking differential staining.

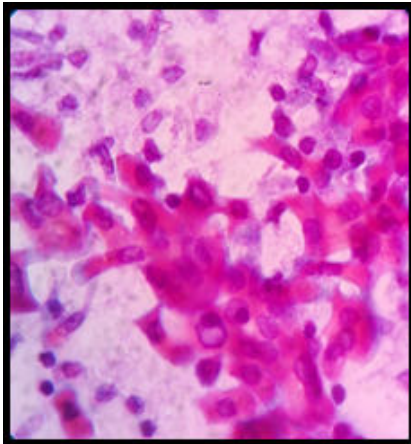


Figure 1:Prosafe Pap stain ,100x

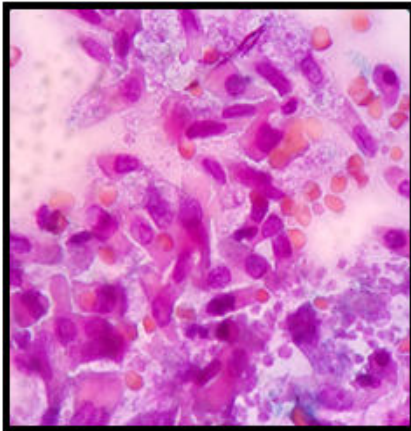


Figure 2: Prosafe Pap stain ,100 x

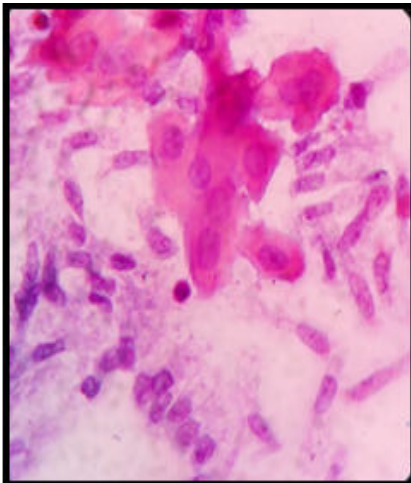


Figure 3:Prosafe Pap stain, 100 x

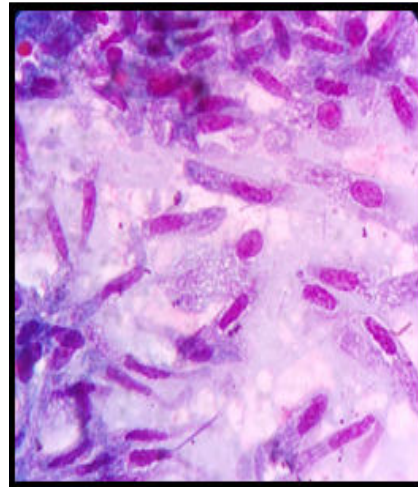


Figure 4:Prosafe Pap stain, 1000 x

Table 3:

CASES= 100				
	Conventional Pap		Prosaf e Pap	
BACKGROUND	N	%	N	%
HEMORRHAGIC	20	20	05	05
CLEAR	80	80	95	95
OVERALL STAINING				
POOR	2	2	0	0
AVERAGE	30	30	10	10
GOOD	68	68	90	90
CELL MORPHOLOGY				
POORLY PRESERVED	3	3	01	01
MODERATELY PRESERVED	25	25	08	08
WELL PRESERVED	72	72	91	91
NUCLEAR CHARACTERISTICS				
SMUDGY CHROMATIN	08	08	02	02
MODERATELY CRISP CHROMATIN	20	20	08	08
CRISP CHROMATIN	72	72	90	90
CYTOPLASMIC DETAILS				
UNSATISFACTORY	05	05	02	02
SUB-OPTIMAL	09	09	03	03
OPTIMAL	86	86	95	95
AIRDRYING ARTEFACTS				
>50%	12	12	00	00
<50%	20	20	02	02
0%	68	68	98	98
UNSATISFACTORY	05	05	02	02
SUB-OPTIMAL	09	09	03	03
OPTIMAL	86	86	95	95
AIRDRYING ARTEFACTS				
>50%	12	12	00	00
<50%	20	20	02	02
0%	68	68	98	98

DISCUSSION

Pap stain remains the traditional and most widely used stain , not only for gynecological cytology , but also for various lesions of other organs. Pap test has been extensively used for early detection of cancerous , precancerous as well as inflammatory lesions world wide. increased turn around time , high cost and difficulty in availability of alcohol has lead to various modifications of Pap stain in different laboratories.The different staining methods of air dried smears are MGG, Jenner –Giemsa and Diff –Qiuck stain , but they did not offer transparency in the study of subtle nuclear features,opacity of nuclei and flatness of image as seen by the Pap stain.

The conventional Pap staining methods proceeds with wet fixation and further staining process together requires minimum 30 minutes. To cut short the time, Kline ,Tao and sato developed rapid Pap stain which required staining time of 5 minutes, 4 minutes and 90 secs respectively. The quality of rapid Pap stain is usually not good , as the cell morphology is not well satisfactory .

To overcome these problems, ultra fast Pap stain was developed by Yang and Alvarez . It is a hybrid of Pap and Romonowsky stains. The staining time is 90 seconds.

In this study, cytomorphology of Prosafe Papanicolaou stain was compared with conventional papanicoaou stain .

Stain quality was evaluated in six parameters , as background, overall staining , cell morphology , nuclear characteristics , cytoplasmic features and air drying artefacts .

Advantages of Prosafe Pap stain compared to conventional Pap:

- Bluing is done in tap water instead of chemical bluing (Scott's bluing agent) .Bluing is best done with tap water pH of 5-7.
- Descending grades of ethanol or two steps of dehydrating ethanol is replaced by one step of 80% isopropyl alcohol.
- As fixation time(1 min) is very less and staining time is 4 mins, therefore very useful for intra operative cytology , rapid assessment of adequacy of samples and rapid diagnosis.
- Background is clear , RBC free and thus helps in better interpretation.
- This is especially useful for smears of vascular organs like thyroid and in identification of Reed Sternberg's cells of Hodgkin's lymphoma.
- Air drying removes artefactual changes seen in wet fixed smears due to poor fixation.
- Because of its cost effectiveness it can be used as suitable alternative to standard pap stain in mass cervical cancer screening in developing countries.

CONCLUSION

Prosafe pap is a simple and fast staining technique for cervical smears with less turn around time .It showed maximum score for all six parameters with crisp nuclear chromatin in 90% of cases and optimal cytoplasmic features in 95% of cases. Lesser time for staining with good morphological quality is the need of the hour in any cytology laboratory.

Prosafe Pap fulfills these parameters equal to or even better than conventional pap technique for cytologic staining.

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