



HAZARD FREE PAP IN ENDOMETRIAL CYTOLOGY

Pathology

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ABSTRACT

Introduction: Pap stain is a universal stain which is used for cytological screening. It is a very reliable technique. There are certain limitations of routine PAP staining like using of ethanol as dehydrating agent which is costly, hazardous and color preservation is not long standing. **Aim:** Our aim was to design a method using a limited amount of ethyl alcohol and it had to be rapid and economical as standard Papanicolaou stain without compromising staining quality. **Materials And Methods:** A total of 200 smears collected from the endometrium of 100 unfixated total hysterectomy specimens, 2 smears from each specimen, received in Pathology department of S Nijalingappa Medical College, Bagalkot. Of these, 100 smears underwent standardization of the Hazard free Pap staining and their counterparts underwent routine Papanicolaou staining. **Results:** The two methods were compared in respect of staining quality, cytoplasmic, nuclear details, time, cost and its ecofriendly feature. Staining time was minimized to 4 minutes from 20 minutes. In Hazard free staining, the nuclear and cytoplasmic staining was optimal in 90 and 93 respectively. **Conclusion:** Hazard free Pap stain provides a suitable, excellent and rapid alternative for cytological screening with minimum cost, lesser time and being ecofriendly by avoiding ethanol.

KEYWORDS

PAP staining, modified PAP

INTRODUCTION

Pap stain is a universal stain used for cytological screening (2). It yields polychromatic, transparent staining with crisp nuclear and cytoplasmic features (5). It is a very reliable technique and is called Pap Smear.

Pap stain is not fully standardized; it comes in several versions, subtly differing in the exact dyes used, their ratios, and timing of the staining procedure. (5).

Various articles describe modifications of Pap staining such as Ultra Fast and Rapid Pap. These modifications have reduced the staining timings and the results of the stained smears are comparable to the routine PAP stain. There are certain limitations of routine PAP staining like using of ethanol as dehydrating agent which is costly and color preservation is not long standing. In India, getting license for acquiring ethanol in large quantity and its annual renewal is herculean task (1).

Our aim was to design a method using a limited amount of ethyl alcohol and it had to be rapid and economical as standard Papanicolaou stain without compromising staining quality. We refer to this modification as Hazard free Pap staining.

MATERIALS AND METHODS

A total of 200 smears collected from 100 endometrium of unfixated total hysterectomy specimens, 2 smears from each specimen, received in Pathology department of S Nijalingappa Medical college, Bagalkot. Of these, 100 smears underwent standardization of the new modification, Hazard free Pap staining and their counterparts underwent routine Papanicolaou staining.

Method of Hazard Free Pap Staining:

1. Fix cytology smear in 80% isopropyl alcohol for 1 minute.
2. Hydrate the smear with water for 30 seconds.
3. Harris hematology for 30 seconds.
4. Wash in alkaline water for 30 seconds.
5. Dip in liquor ammonia.
6. Dehydrate the smear with 80% isopropyl alcohol for 30 seconds.
7. Stain in equal part of OG-6 and EA 36 for 30 seconds.
8. Wash in 80% isopropyl alcohol for 30 seconds.
9. Dry and mount.

After staining both Hazard free Pap and Papanicolaou stained smears screened together by the first and second authors, and the Hazard free Pap stained smears were screened separately, without any comparison

or bias, by the third and fourth authors. The Hazard free Pap smears were compared with Papanicolaou smears using various parameters: Transparency of cytoplasm, nuclear details, staining quality, turnaround time and preservation of stain quality.

Table:1 Scoring System Used In Assessment of Staining

PARAMETER	SCORE 1	SCORE 2
OVER ALL STAINING	GOOD	AVERAGE
CELL BORDER	DISTINCT	INDISTINCT
CYTOPLASMIC DETAILS	SATISFACTORY	UNSATISFACTORY
NUCLEAR DETAILS	DISTINCT	INDISTINCT
CHROMATIN	CRISP	HAZY
WBC, RBC, PARASITE, NECROSIS	PRESENT	ABSENT

The maximum score was 18 for a single case, it was considered into account all six parameters.

RESULTS

A total number of 100 specimens and for each specimen two smears were taken. One slide was stained using conventional technique and the other was stained using the Hazard free Pap staining techniques.

The staining quality of Hazard free Pap technique was better than conventional PAP stain. 93 out of 100 cases showed good transparency of cytoplasm. The rest were suboptimal due to thick smears. Nuclear details like margins, nucleolus and chromatin pattern were optimum in most of cases like 90 out of 100 cases.

Staining reactions of non-epithelial cells like RBC, WBC were well preserved than the standard Papanicolaou stain. The staining quality remained well preserved for 8 months without any fading. The cytoplasmic stain was optimal in 93 out of 100 cases. Rest of 7 cases, showed suboptimal stain because the stain could not penetrate the thick part. In 90 out of 100 cases, nuclear staining showed optimal feature. Rest of cases, the nuclear staining was pale and suboptimal. The staining quality was preserved for 8 months.

Table:2 Comparison Between Conventional And Hazard Free Pap

CASES 100	
CONVENTIONAL PAP (100)	HAZARD FREE PAP (100)

OVERALL STAINING		
GOOD	80	98
AVERAGE	20	02
CELL BORDER		
DISTINCT	71	97
INDISTINCT	29	03
CYTOPLASMIC DETAILS		
SATISFACTORY	83	93
UNSATISFACTORY	17	07
NUCLEAR DETAILS		
DISTINCT	76	90
INDISTINCT	24	10
CHROMATIN		
CRISP	70	96
HAZY	30	04
WBC,RBC,PARASITE, NECROSIS		
PRESENT	60	90
ABSENT	40	10

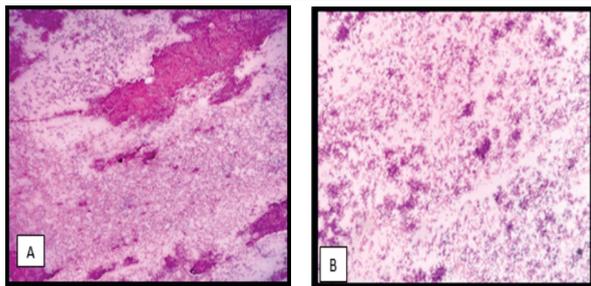


Figure 1: A & B shows 10x comparison between Hazard free Pap stain and conventional Pap.

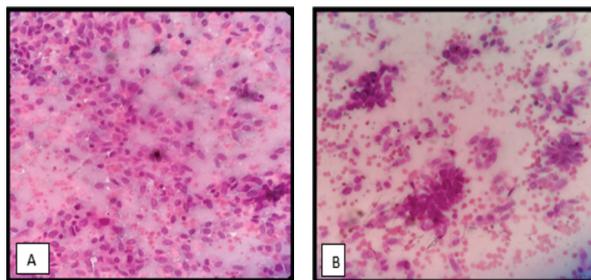


Figure 2: A & B shows 40X comparison between Hazard free Pap and Conventional Pap

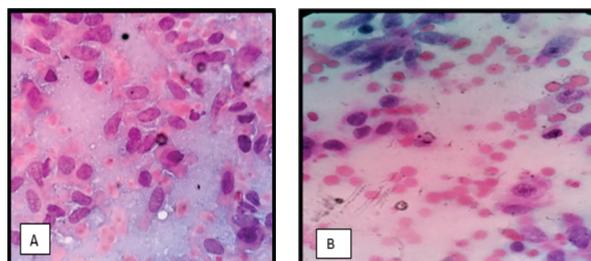


Figure 3: A&B shows 100 X comparison between Hazard free Pap and conventional Pap

DISCUSSION

Papanicolaou stain underwent various modifications. Even after various modifications, the problem of usage of expensive ethyl alcohol has not been completely resolved so far (1).

An important aspect of a diagnostic test is quick turnaround time. The time taken by the Hazard free Pap method to stain a smear is 4 minutes as compared to 20 minutes by the Papanicolaou method.

Hazard free Pap staining is novel modification of Papanicolaou stain as it is not only rapid but also uses limited amount of isopropyl alcohol, there by stain is ecofriendly. It is not compromising quality. The result of this study demonstrate that Hazard free Pap stain provide excellent screening and rapid diagnosis. Table 3 & 4 shows the comparison between conventional Pap and Hazard free Pap staining.

Table.3 Conventional Pap Staining Procedure

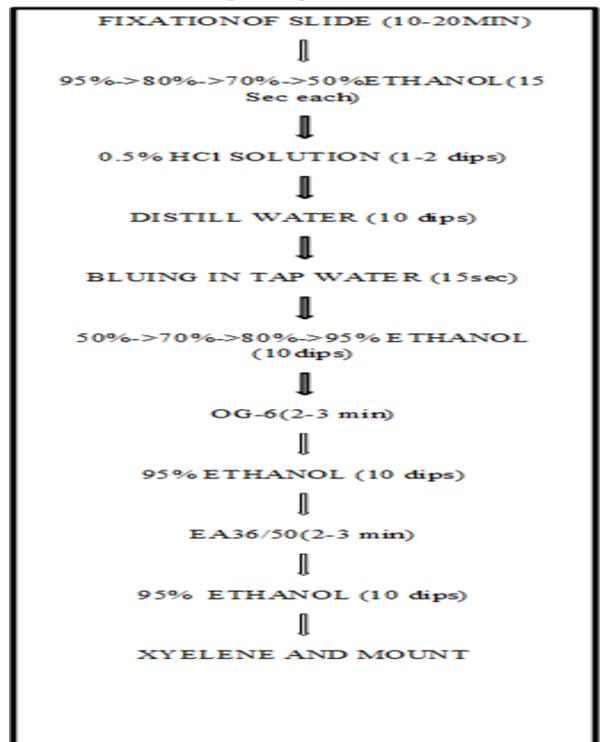
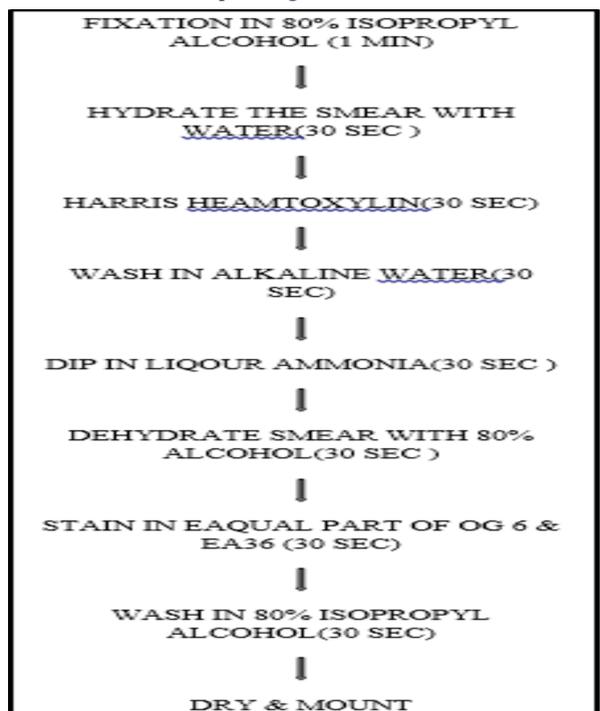


Table.4 Hazard Free Pap Staining Procedure



CONCLUSION:

Hazard free Pap stain provides a suitable, excellent and rapid alternative for cytological screening with minimum cost and lesser time.

Over all, the use of alcohol is minimum. The stain preservation is good, ecofriendly and no biohazards. It can be considered as a suitable alternative to standard Papanicolaou stain.

REFERENCES

1. Dighe SB, Dulhan A, Pathuthara S, Chinoy R. Is it economical to switch to Rapid, Economic, Acetic acid, Papanicolaou stain? Acta Cytol 2006;50(6):643-646.
2. Biswas Ranu R, Paral CC, Dey R et al. Rapid Economic, Acetic Acid ,Papanicolaou Stain (REAP)-Is It suitable alternative to standard PAP stain. Al Ameen J MedSci.2008; 1(2):99-103.

3. Asthana A, S.A. Comparison of the routine Papanicolaou staining technique with the rapid, economic, acetic acid, Papanicolaou (REAP) technique.IJMDS.2014 Jul;3(2):484-489.
4. Raju K. Evolution of Pap Stain. Biomedical Research and Therapy .2016 Feb; 3(2):490500.
5. Hussein GH, Hameed IF et al. Modified Rapid Papanicolaou Stain As A Suitable Alternative Stain To Standard Papanicolaou Stain.wjpr 2017; 6(2):242-249.