

# Comparative Study of Rapid Economic Acetic Acid Papanicolaou Stain and Papanicolaou Stain

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## ABSTRACT

### BACKGROUND

The Papanicolaou stain is used in Pap smear test for screening women for cervical cancer. As this test is used extensively, there is always a need for newer and better stains that would be equivalent to the traditional Pap stain in terms of staining quality and at the same time having the advantages of quicker staining and low cost. Rapid Economic Acetic Acid (REAP) is one such stain. We wanted to compare the staining of traditional Papanicolaou stain with Rapid Economic Acetic Acid (REAP) stain for the Pap smear test.

### METHODS

This was a prospective study done over a period of four months in the Department of Pathology, at PSIMS, Vijayawada. A total of 250 Pap smear samples were studied. Two slides for each woman who underwent the Pap smear test, were collected. One slide was stained with the traditional Pap stain and the other was stained with REAP stain and both the slides were compared in terms of staining quality, turn-around time, preservation of slides and cost.

### RESULTS

The differentiation and transparency of the cytoplasm of REAP were optimal in 97 % smears. The staining quality was almost similar for both the stains. However, REAP stain required lesser staining time (3 minutes) and costed one fourth of the Pap stain.

### CONCLUSIONS

REAP is a rapid, cost effective alternative to Papanicolaou stain for Pap smear test. It provides excellent staining and is less expensive. It also improves the turn-around time of the Pap smear test.

### KEYWORDS

Cervical Cancer Screening, REAP, Pap Smear

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**BACKGROUND**

Conventional Papanicolaou stain (PAP) staining is commonly used to detect premalignant / malignant changes in the exfoliated cervical cells. The cytologic screening is highly effective in preventing cervical cancer because most of the malignancies are preceded by long standing precancerous lesions, which may exist for several years and shed abnormal cells.<sup>1</sup> Papanicolaou stain, a polychromatic cytological staining technique for cervical smears was first developed by George Papanicolaou in 1942 and was subsequently modified by him in 1954 and 1960. It is a standard and reliable technique used for screening of women for cervical cancer. The entire procedure is simply known as Pap smear test.<sup>2</sup> cervical smear screening has greatly contributed in reducing morbidity and mortality due to carcinoma cervix and is well - established globally in screening programmes for the past fifty years.

Cervical cancer is the second most common cancer in women world-wide<sup>3</sup> and is very common in India too. In India, cervical cancer screening is not a mandatory test but is done widely as part of screening camps organized by various medical institutions and Non-Governmental Organizations.

The cervical smears that are collected are stained by the traditional Pap stain through various stains and reagents. PAP staining uses alcohol at various steps, which is costlier and the procedure takes longer time of about twenty minutes. To overcome these disadvantages, attempts for less expensive and rapid staining procedure, but at the same time not compromising on the quality were done. The various methods which were tried included Ultra - fast staining, Rapid PAP, Quick PAP staining and REAP stain. Rapid economic acetic acid Papanicolaou stain (REAP) is one of the alternative method to routine PAP stain. REAP procedure was first introduced by Dr. Roshni Chinoy, Tata Memorial hospital, Mumbai.<sup>4</sup> PAP stain uses alcohol at various stages of staining which is expensive and also difficult to procure. REAP Stain does not use alcohol and is replaced by acetic acid and hence is cheap and rapid.

We wanted to compare the staining of traditional Papanicolaou stain with Rapid Economic Acetic Acid (REAP) stain for the Pap smear test, with regard to quality of staining, duration of staining, cost effectiveness, preservation of staining, advantages and disadvantages.

**METHODS**

The study was approved by the Institutional Ethics Committee. This was a prospective study done over a period of four months from October 2019 to February 2020, in the Department of Pathology, at Pinnamaneni Siddhartha Institute of Medical Sciences and Research Foundation, Vijayawada, India. A total of 250 women underwent the cervical smear test in the study period. The cervical smears were collected in the OPD of Department of Gynaecology

and were submitted to the Department of Pathology, Cytology section for processing.

Two Pap smears were obtained from each woman instead of one. The smears were fixed in methanol for 20 - 30 min and stained simultaneously by conventional PAP and REAP staining methods in the Department of Pathology.

1	Distilled water	Rinse
2	Gills Hematoxylin	8 - 10 min
3	Wash in tap water	1 - 2 min
4	Differentiate in acid alcohol	1 - 2 dips
5	Lithium carbonate	2 - 3 dips
6	Distilled water	Rinse
7	70 % Alcohol	few seconds
8	95 % Alcohol	few seconds
9	OG6	1 - 2 min
10	3-changes of ethyl alcohol	few sec
11	EA 36	1 - 2 min
12	3-changes of 95 % alcohol	few sec
13	Absolute alcohol-dehydration	1 - 2 dips
14	Xylene - clear	1 - 2 dips
15	Mount	

**Table 1. PAP Staining Procedure**

Nucleus appears blue and cytoplasm appears polychromatic (red, orange, blue, green, pink)

1	1% Acetic acid	10 dips
2	Gills Hematoxylin (60 degree centigrade)	10 dips
3	Tap Water	10 dips
4	1 % Acetic acid	10 dips
5	OG 6	10 dips
6	1% Acetic acid	10 dips
7	EA36	10 dips
8	1 % Acetic acid	10 dips
9	Methanol	10 dips
10	Xylene	10 dips

**Table 2. Procedure of REAP Staining**

Blot after every step

Mount- Canada Balsam / DPX

Results: Nucleus appears blue and cytoplasm appears polychromatic (red, blue, orange, green, brown).

**RESULTS**

A total of 250 paired smears stained by conventional Papanicolaou and REAP stain were examined by two pathologists. The slides were assessed for optimal and suboptimal nuclear staining, optimal and suboptimal cytoplasmic staining. The average time taken for routine PAP and REAP staining was compared. The effective cost of PAP and REAP staining procedures was calculated and compared. The preservation of colour intensity of the smears stained by PAP and REAP were also compared over four months observation period with periodic checks at monthly intervals.

Table 3 compares the cytoplasmic staining quality of the REAP and PAP smears. The nuclear details and the chromatin pattern were compared between the two. REAP smears showed optimal nuclear staining in 95 % smears and sub - optimal staining in 05 % smears. The differentiation and transparency of the cytoplasm of REAP were optimal in 97 % smears. In 03 % smears the cytoplasmic stain penetration was sub - optimal, especially in areas of overlapping cell clusters. Time taken for PAP staining was 20 min whereas for REAP staining it took only 03 min. All the slides were observed for preservation of staining and it was found to be excellent for 4 months. Similarly cost of the REAP staining was found to be very less and it was one fourth of the traditional Pap stain.

Sl. No.	Features	Pap Stain	Reap Stain
1	Nuclear staining		
	a) optimal	100 %	95 %
	b) sub-optimal	----	5 %
2	Cytoplasmic staining		
	a) optimal	98 %	97 %
	b) sub-optimal	02 %	03 %
3	Staining time	20 min	3 min.
4	Preservation (4 months)	Excellent	Excellent
5	Cost / smear	More cost	Less cost (1 / 4 of Pap stain)

**Table 3. Features of Both PAP and REAP Staining**

## DISCUSSION

Papanicolaou stain was first introduced by George Papanicolaou who is also known as the Father of Cytology. It is the best stain for cytological Pap smears as it yields polychromatic, transparent staining reaction with crisp nuclear and cytoplasmic features. It is most commonly and routinely used stain in many institutions and laboratories. It is very effective but consumes more amount of ethanol and it takes relatively longer time of about twenty minutes. Modifications of PAP staining have been done earlier to overcome the cost and long duration.

The various methods tried are, Ultra - fast staining, Rapid PAP, Quick PAP staining. These alternative methods are rapid and take 90 seconds time for staining, but use expensive ethyl alcohol. In India, purchase of alcohol in bulk even for laboratory purpose requires license which has to be renewed every year. This becomes a cumbersome task. So any viable alternative staining method that obviates the need of alcohol is welcome. In REAP, all the steps that use ethyl alcohol are substituted by 1 % acetic acid which is a mild dehydrating agent.<sup>5</sup>

The present study included 250 paired slides stained by conventional PAP and REAP methods. The quality of staining was assessed by taking into consideration cell borders, cytoplasmic staining, nuclear borders and chromatin staining and were categorized as optimal and sub-optimal. Present study showed the differentiation and transparency of the cytoplasm of REAP were optimal in 97 % smears. In 03 % smears the cytoplasmic stain penetration was sub - optimal, especially in areas of overlapping cell clusters. In a similar study by Dighe BS et al<sup>5</sup> they found that with the REAP method, cytoplasmic and nuclear staining were optimal in 181 and 192 cases, respectively. Tellapuram V et al<sup>6</sup> in their study observed good cytoplasmic transparency and optimal nuclear details in REAP stained smears when compared to the conventional PAP stain.

Dighe BS et al<sup>5</sup> in their study of 200 cases showed that REAP stained smears had sub - optimal cytoplasmic and nuclear staining of 19 and 8, respectively. Whereas PAP stain smears did not show any sub - optimal cytoplasmic or nuclear staining. The present study of 250 cases revealed that REAP stained smears showed sub-optimal cytoplasmic staining in 3 % slides and sub-optimal nuclear staining in 5 % slides. Conventional PAP showed sub-optimal cytoplasmic and nuclear staining in 2 cases.

Stain preservation was excellent for four months in the present study, whereas, in the study done by Biswas et al,<sup>7</sup> it showed good preservation for 6 months. In the study done by Dighe BS et al<sup>5</sup> the staining quality remained good in all

the smears for more than two years. Gachie et al<sup>8</sup> did not include this parameter in their study.

All the above studies concluded that REAP stain showed near similar results as conventional PAP method. Our observations compare well with the above studies.

Sl. No.	Parameter	Tellapuram V et al <sup>6</sup>		Dighe BS et al <sup>5</sup>		Present Study	
<b>Nuclear stain</b>							
1	a. Optimal	60 %	91 %	100 %	96 %	100 %	95 %
	b. Sub-optimal	40 %	9 %	-	4 %		5 %
2	Turnaround time	30 min	07 min	20 min	03 min	20 min	0 3 min
3	Preservation	Excellent (2 Years)		Excellent (2 years)		Excellent	
4	Cost	4\$	1\$	240 Rs	60 Rs	100	25 Rs

**Table 4. Comparative Studies**

## CONCLUSIONS

REAP is a rapid, cost effective alternative to Papanicolaou stain for Pap smear test. It provides excellent staining and is less expensive. Final interpretation and reporting of the smears is not compromised with REAP. It also improves the turn-around time of the Pap smear test.

Financial or Other Competing Interests: None.

## REFERENCES

- [1] Sato M, Taniguchi E, Kagiya T, et al. A modified rapid Papanicolaou stain for imprint smears. *Acta Cytol* 2004;48(3):461-462.
- [2] Bibbo M. Cytopreparation techniques. In: *Comprehensive Cytopathology. (Rapid pap staining)*. 2<sup>nd</sup> edn. Philadelphia: W.B. Saunders Company, 1997: p. 635
- [3] WHO / ICO information center on Human papilloma virus and related cancers in India. Summary report 2009.
- [4] Yang GC, Alvarez II. Ultrafast Papanicolaou stain. An alternative preparation for FNAC. *Acta Cytology* 1995;39(1):55-60.
- [5] Dighe SB, Ajit D, Pathuthara S, et al. Papanicolaou stain: Is it economical to switch to rapid, economical, acetic acid, papanicolaou stain? *Acta Cytology* 2006;50(6):643-646.
- [6] Tellapuram V, Yogesh. The merits of rapid, economic acetic acid, papanicolaou stain (REAP) over papanicolaou stain (PAP) technique in cervical cytology. *Int J Res Med Sci* 2017;5(8):3334-3339.
- [7] Biswas RR, Paral CC, Dey R, et al. Rapid economic acetic acid papanicolaou atain (REAP) - Is it suitable alternative to standard PAP stain? *Al Ameen J Med Sci* 2008;12:99-103.
- [8] Gachie RN, Muchiri LW, Ndungu JR. A Comparison of modified and standard Papanicolaou staining methods in the assessment of cervical smears at Kenyatta National Hospital. *East African Med J* 2011;88(7):244-250.