

COMPARATIVE STUDY OF PAPANICOLAOU STAIN [PAP] WITH RAPID ECONOMIC ACETIC ACID PAPANICOLAOU STAIN (REAP) IN CERVICAL CYTOLOGY

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ABSTRACT: INTRODUCTION: The present comparative study of PAP and REAP stain was carried out to assess the quality of staining, duration of staining, cost effectiveness and preservation of staining. **MATERIALS AND METHODS:** A total of 200 samples were studied over a period of 2 years. The pap smears were obtained from the obstetrics and gynecology out-patient department, fixed in 95% ethyl alcohol and stained simultaneously by both the methods in the department of cytology. **RESULTS:** Pap stain showed 2 cases (1%) of sub optimal cytoplasmic and nuclear staining whereas REAP stain showed 6 cases (3%) of suboptimal cytoplasmic staining and 4 cases (2%) of suboptimal nuclear staining. A batch of ten slides each was stained by conventional PAP method and REAP method and the time taken was 35 min and 7 min respectively. The cost per slide worked out to be Rs. 40 in case of conventional PAP method and Rs. 10 in case of REAP method. Slides stained by both methods showed excellent preservation for 2 years. **CONCLUSIONS:** The REAP staining method for study of cervical smears in cancer screening programmes is a simple and technician friendly protocol with minimum use of alcohol, that does not compromise on staining quality and diagnostic standards. It can be easily adapted as a viable alternative to conventional PAP method which is time consuming and expensive for mass screening of cervical cancer in limited resource setting like India. We recommend this procedure for all mass cervical cancer screening programmes.

KEYWORDS: Conventional PAP stain, REAP stain, Cervical cytology.

INTRODUCTION: Papanicolaou stain, a multichromatic cytological staining technique was developed by George Papanicolaou in 1942 and subsequently modified by him in 1954 and 1960.¹ It is a very reliable technique used for cervical cancer screening in gynecology. The entire procedure is known as PAP smear.² PAP stain paved the way for screening and early diagnosis of precancerous lesions of cervix and contributed greatly in reducing morbidity and mortality due to carcinoma cervix.

Due to increasing burden of cancer screening programme, PAP stain is proving to be less cost effective, cumbersome and time consuming. Hence, the search for a less expensive and rapid stain, but at the same time not compromising on the quality.

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.

MATERIALS AND METHODS: Cervical cytology smears were obtained from obstetrics and gynecology out-patient department, KIMS, Narketpally, and staining done in the department of cytology. A set of 2 slides was obtained for each patient and one stained by PAP method and the other by REAP method simultaneously.

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PAP SMEAR COLLECTION:

- Patient was made to lie down in lithotomy position.
- Cusco's speculum was introduced in the vagina.
- Cervix identified.
- Ayres spatula was introduced through Cusco's speculum into the os [Squamo columnar junction].
- The spatula was rotated 360 degrees and the smear was collected, spread on glass slide and placed in a fixative of 95% ethyl alcohol.
- Slides were sent to cytology lab for staining.

CONVENTIONAL PAP TECHNIQUE:

1. Transfer slides directly from alcohol-ether fixative without drying, to 80% alcohol, and bring down through 70% and 50% alcohol to distilled water.
2. Stain in Harris hematoxylin for 4 min.
3. Rinse briefly in distilled water.
4. Dip in 0.25% HCL in 50% ethanol about six times (20-60sec).
5. Place in running tap water for 6 min.
6. Rinse in distilled water and run through 50%, 70%, 80%, 95% alcohol.
7. Stain in OG-6 for 2 min.
8. Rinse in two changes of 95% alcohol.
9. Stain in EA36/EA 50 for 2 min.
10. Rinse in three changes of 95% alcohol. Dehydrate in absolute alcohol, followed by equal parts absolute alcohol and xylol; clear in xylol and mount.

RAPID ECONOMIC ACETIC ACID PAP STAIN TECHNIQUE was followed (as given by Biswas et al).³

1% ACETIC ACID	10 dips
Harris Hematoxylin	10 dips
[Preheated 60C]	
Tap water	10 dips
1% acetic acid	10 dips
OG-6	10 dips
1% acetic acid	10 dips
EA-50	10 dips
1% acetic acid	10 dips
Methanol	10 dips
Xylene	10 dips

Blotting was done after each step.

Mount in D. P. X.

All the alcohol steps in Conventional PAP were replaced by 1% Acetic acid in REAP.

METHOD OF ANALYSIS OF STAINING:

Slides after staining by PAP and REAP method were analyzed.

Cytoplasmic staining – Optimal Suboptimal.

Nuclear staining – Optimal Suboptimal.

RESULTS: A total of 200 paired smears stained by conventional papanicolaou and REAP stain were examined in the study. The minimum age of patient screened was 20 years and maximum 60 years. The majority were in the age group of 31-40 years. The mean age was 40 years. (Table 1).

The staining quality was compared for both the stains taking into criteria the cell and cytoplasmic border, cytoplasmic staining, nuclear borders and chromatic staining (Table 2).

2(1%) of the smears had indistinct cell borders and 198(99%) had distinct cell borders in the conventional PAP method.

In REAP 4(2%) of the smears had indistinct cell borders and 196(98%) had distinct cell borders.

In conventional PAP2 (1%) showed unsatisfactory cytoplasmic staining and 198(99%) showed satisfactory cytoplasmic staining whereas REAP showed 6(3%) of unsatisfactory cytoplasmic staining and 194(94%) showed satisfactory cytoplasmic staining.

2(1%) of smears had indistinct nuclear borders and 198(99 %) had distinct nuclear borders, in smears stained by both PAP and REAP.

In PAP chromatin staining was hazy in 2(1%) and distinct in 198(99%) in REAP chromatin staining was hazy in 4(2%) and distinct in 196(98 %).

Out of 200 paired slides stained by PAP and REAP, PAP showed 2 cases of suboptimal cytoplasmic and nuclear staining whereas REAP showed 6 cases of suboptimal cytoplasmic staining and 4 cases of suboptimal nuclear staining. (Table 3)

One batch of 10 slides was stained by conventional PAP and REAP method and the time taken was 35 min and 7 min respectively. The cost per slide worked out to be Rs. 40 in case of conventional PAP method and Rs. 10 in case of REAP.

Slides stained by both methods showed excellent preservation for 2 years (Table 3).

DISCUSSION: PAP stain results in well stained nuclear chromatin, differential cytoplasmic counter staining and cytoplasmic transparency⁴ and hence, has been very popular for cervical cancer screening programmes all over the world for more than 50 years.

Cancer of the cervix has been the most important cancer in women in India. Based on the data of population based cancer registries the number of new cases in India during 2007 was 90,708.⁵ It is the second most common cancer in women world-wide.⁶

PAP stain paved the way for screening and early diagnosis of precancerous lesions of cervix and contributed greatly in reducing the morbidity and mortality due to carcinoma cervix.⁷

The procedure is complex involving various steps and different timings and costly to run because of the use of ethyl alcohol in many steps ⁸ hence, the need for a viable alternative. REAP is one such method.⁹

In our country purchase of ethyl alcohol in bulk requires license. Obtaining license and its annual renewal is an arduous task. REAP which does not use alcohol comes to our rescue.⁹ In REAP, the various ethyl alcohol steps are substituted by 1% acetic acid which is a mild dehydrating agent.¹⁰

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The present study included 200 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 suboptimal.

In case of Biswas et al,³ it was found that REAP showed suboptimal cytoplasmic staining of 10 cases and nuclear suboptimal staining of 5 cases out of total cases of 110 studied, whereas, PAP showed a suboptimal cytoplasmic staining of 20 and suboptimal nuclear staining of 10 (Table 4).

Similarly Gachie et al,¹¹ studied 159 cases, showed cytoplasmic and nuclear suboptimal staining of 21 cases stained by REAP method whereas conventional PAP method had shown a suboptimal cytoplasmic and nuclear staining of 29 cases. Both the above studies concluded that REAP stain showed better results than conventional PAP method. (Table 4)

Dighe et al,¹⁰ in their study of 200 cases showed that REAP stained smears had suboptimal cytoplasmic and nuclear staining of 19 and 8 respectively. Whereas PAP stain did not show any suboptimal cytoplasmic or nuclear staining.

The present study of 200 cases revealed that REAP stained smears showed suboptimal cytoplasmic staining of 6 cases and suboptimal staining of 4 cases. Conventional PAP showed suboptimal cytoplasmic and nuclear staining in 2 cases.

Stain preservation was excellent for 2 years in the study of Dighe et al,¹⁰ and present study whereas in the study done by Biswas et al,³ it showed good preservation for 6 months only.

Gachie et al,¹¹ did not include this parameter in their study. (Table 4)

Biswas et al,³ study, REAP proved cheaper to conventional PAP by four times. Turn-around time for REAP was only 3 min where as conventional PAP took 20 min. Dighe et al,¹⁰ also showed similar results in case of turn-around time and cost effectiveness. (Table 5)

In case of Gachie et al,¹¹ turn-around time was same as the above two studies for both the stains but the cost for REAP stain was reduced by 6 times. Present study showed reduction of cost for REAP stain by 4 times and turn-around time was reduced by 5 times. (Table 5)

Izhar et al,¹² in their study concluded that REAP stain cannot be used for routine staining in a tertiary care hospital for research purpose due to poor preservation of staining quality after 6 months.

Other additional findings noted in our study were:

- A. The background of smears stained by REAP was extremely clear without any debris when compared with PAP stained slides.
- B. The staining characteristics of candida and trichomonas vaginalis in REAP stained smears were much better than PAP.

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Age (Yrs)	No. of Patients	%
20-30	69	34.5
31-40	81	40.5
41-50	30	15
51-60	20	10
TOTAL	200	100

Table 1: Age Distribution of The Cases N=200

Maximum number of cases are in age group 31 - 40yrs (40.5%).

Parameter	Conventional PAP	%	REAP	%
Cell / Cytoplasmic borders				
Distinct	198	99	196	98
Indistinct	2	1	4	2
Cytoplasmic staining				
Satisfactory	198	99	196	98
Unsatisfactory	2	1	4	2
Nuclear-Border				
Distinct	198	99	196	98
Indistinct	2	1	4	2
Chromatin staining				
Distinct	198	99	196	98
Hazy	2	1	4	2

Table 2: Cytomorphologic Features N=200

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Quality of nuclear border staining was same for both PAP and REAP, whereas quality of cell border, cytoplasmic staining and chromatin staining of REAP was slightly less than PAP.

Procedure	Cytoplasmic Stain		Nuclear Stain		Smear Preservation (2 yrs)	Turn-around time	Cost per smear
PAP	198	2	198	2	Excellent	35 min	Rs. 40
REAP	194	6	196	4	Excellent	7 min	Rs. 10

Table 3: Results of Papanicolaou and Reap Methods N=200

Quality of REAP staining was as good as PAP whereas turn-around time and cost per smear in REAP were considerably reduced. Preservation was excellent in both cases for two years.

Authors		Cytoplasmic Stain		Nuclear Stain		Smear Preservation
		Optimal	Sub optimal	Optimal	Sub optimal	
Dighe et al N= (200) (2006)	PAP	200 (100%)	-	200 (100%)	-	Excellent (2 yrs)
	REAP	181 (90.5%)	19 (9.5%)	192 (96%)	8 (4%)	Excellent (2 yrs)
Biswas et al N = (110) (2008)	PAP	90 (81.8%)	201 (18.2%)	100 (90.9%)	10 (9.1%)	Excellent (6months)
	REAP	100 (90.9%)	10 (9.1%)	105 (95.4%)	5 (4.6%)	Excellent (6months)
Gachie et al N= (159) (2011)	PAP	130 (81.7%)	29 (18.3%)	130 (81.7)	29 (18.3)	-
	REAP	138 (86.7%)	21 (13.3%)	138 (86.7%)	21 (13.3%)	-
Present Study N=200 (2013)	PAP	198 (99%)	2 (1%)	198 (99%)	2 (1%)	Excellent (2 yrs)
	REAP	194 (97%)	6 (3%)	194 (97%)	6 (3%)	Excellent (2 yrs)

Table 4: Comparison of Quality of Staining and Smear Preservation, of Pap and Reap with Other Authors N=200

- Quality of staining - Biswas et al, Gachie et al REAP fared better than PAP whereas in Dighe et al and present study PAP fared slightly better than REAP.
- Smear preservation - Present study and Dighe et al showed excellent preservation for 2 yrs. Biswas et al showed excellent preservation for 6 months, Gachie et al did not include this parameter.

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Authors		Time (min)	Cost per slide (Rs.)
Dighe et al N= (200)	PAP	20	240
	REAP	3	60
Biswas et al N= (110)	PAP	20	100
	REAP	3	25
Gachie et al N= (159)	PAP	20±0.5	123
	REAP	3±0.5	19
Present Study N=200	PAP	35	40
	REAP	7	10

Table 5: Comparison of Cost and Time, of Pap and REAP with Other Authors N=200

- Dighe et al, Biswas et al, Gachie et al time taken for PAP and REAP was 20 min. and 3 min. respectively and cost per slide for REAP reduced by 4 to 6 times.
- Present study the time taken for PAP and REAP was 35 and 7 min. respectively. Cost per slide was reduced by 4 times.

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