

Rapid Economic, Acetic Acid, Papanicolaou Stain (REAP) - Is it suitable alternative to standard PAP stain?

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Abstract: The universal stain for cervical cytological screening is Papanicolaou stain which has been used in different laboratories with many modifications. *Aims:* The study is designed to search for a superior and improved qualitative staining technique which is cheaper but rapid in cancer screening by cytology. The modified technique is referred as Rapid, economic, acetic acid Papanicolaou stain (REAP). *Material & methods:* 220 PAP smears from 110 patients (2 per subject) were collected . One set of smears was stained by conventional Papanicolaou stain & the other set by REAP stain. Pre- Orange G 6 & post- Orange G 6 and post- EA50 ethanol baths in REAP stain were replaced by 1% acetic acid. Tap water was used instead of Scott's tap water to reduce cost. Hematoxylin was preheated in waterbath to 60° C before staining for rapid penetration. Methanol was used for final dehydration. *Results:* The two methods were compared in respect of optimal cytoplasmic & nuclear staining, stain preservation, cost & total time for the procedure. In REAP technique, cytoplasmic & nuclear staining was optimal in 100 & 105 cases respectively. The cost was reduced to 25% due to limited alcohol use. The staining-time was minimised to 3 minutes. *Conclusion:* REAP stain, in comparison to conventional Papanicolaou, provides a suitable, excellent & rapid alternative for cytological screening with minimum cost. The stain preservation is also good in REAP method.

Keywords: Papanicolaou smear, cervical cancer screening, REAP stain.

Introduction

The universal stain for cervical cytological screening is Papanicolaou stain. It yields a polychromatic, transparent staining reaction with crisp nuclear and cytological features. Most laboratories use commercial stains and each will consider their modification of the original technique to be the optimum [1] However it utilizes a considerable amount of ethyl alcohol and takes about 20 minutes. In rapid PAP technique [2], staining is achieved in 90 seconds but it requires a substantial volume of ethanol which is expensive. In India a laboratory needs a license for acquiring ethanol in bulk quantity and obtaining a license and its renewal is a difficult task. Moreover, the concentration of the supplied alcohol is sometimes questionable. Therefore, we are in search of a standardised rapid method suitable for cytological screening of cervix. The modified technique is referred as Rapid, economic, acetic acid Papanicolaou stain (REAP). *Aims of the study:* (i) to design a method that requires hardly any alcohol during staining of cytology and thereby to reduce the expense. (ii) to search for staining of cytology smear that is qualitatively as good as standard Papanicolaou stain for screening of cancer.

Materials and Methods:

Total 220 smears from 110 patients ,attending OPD, Department of Gynaecology , N.R.S. Medical College & Hospital were collected from June, 2007 to August,2007 .Two smears from each patient were collected and stained by standard Papanicolaou stain[3] and REAP method of stain separately.

Only in the first step for fixation and in last step for dehydration, absolute alcohol was used which is same as standard Papanicolaou stain [3].

Methods for REAP stain:

1% acetic acid	10 dips
Harris's Haematoxylin, preheated 60° C	10 dips
Tap water	10dips
1% acetic acid	10 dips
OG-6	10dips
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 by D.P.X.

Total time for staining in the present method was 3 minutes (c.f. Conventional PAP stain of 20 minutes).In Papanicolaou stain, fixed smears are passed through a series of descending grade of ethyl alcohol before nuclear staining. These ethyl alcohol grades are replaced by single 1% Acetic acid step. Harris's hematoxylin is used in both methods for nuclear staining but the time is 1 minute in conventional PAP stain and in REAP it is reduced to 10 dips as the stain is preheated to 60°C.Heating of hematoxylin is done in waterbath to 60°C before staining for rapid penetration[2]. In Standard PAP stain, the blueing agent is Scott's tap water which is replaced by ordinary tap water in REAP stain where the time is also reduced. Before staining with Orange G 6 the two changes of dehydrating Ethyl alcohol grades are replaced by 1% Acetic acid in REAP. The cytoplasmic stains are OG6 and EA50 are same in both methods except the timespent. In both, staining time is reduced from 3 minutes to a few seconds. Two changes of 95%ethyl alcohol with Standard PAP stain after OG6 are replaced by 1% Acetic acid (10dips).In standard PAP stain final dehydration is done by two changes of absolute alcohol. In REAP the smears are washed in 1% Acetic acid & final dehydration by methanol 10 dips. With PAP stain clearing is done by one change of alcohol-xylene followed by 2 changes of xylene

(10 dips each).In REAP clearing is done by single change of xylene (10 dips)
 All the REAP and PAP stained smears were screened by senior pathologists of different units of our department and screened separately without any comparison and bias.REAP smears are compared with conventional PAP smears using following various parameters

Results:

Total no. of patients --- 110

Total no. of smears ---- 220

The staining quality of REAP smears is better than conventional PAP stain.Table-1 compares the staining quality of the REAP & Papanicolaou stain.100 smears out of 110 cases show good transparency of cytoplasm. The rest are suboptimal due to thick smears.Nuclear details like margin, nucleolus and chromatin pattern were optimum in most of the cases,105 out of 110.Staining reaction of non-epithelial cells like RBC ,WBC,bacteria are well preserved as found in standard papanicolaou stain (Fig-1).The staining quality remains well preserved for 6 months without any fading. The cost is minimum in comparison to the total cost of conventional PAP stain.

Table-1: Results of Papanicolaou and REAP methods:

Procedure	Cytoplasmic stain		Nuclear stain	
	Optimal	Suboptimal	Optimal	Suboptimal
PAPANICOLAOU No of smears- 110	90	20	100	10
REAP No. of smears- 110	100	10	105	5

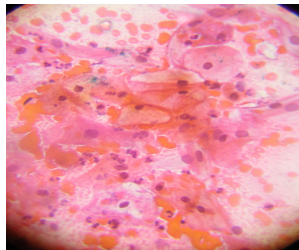


Fig. 1 : REAP stained smear shows mature Superficial cell, intermediate cell with inflammatory cells in the background.(HP)

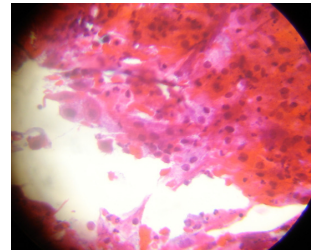


Fig. 2: REAP stained show few moderate dysplastic cells, mature superficial cells and intermediate cells.(HP)

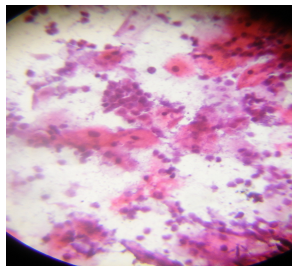


Fig-3 : REAP stained smear shows clumps of endocervical cells, Superficial cells & intermediate cells.(HP)

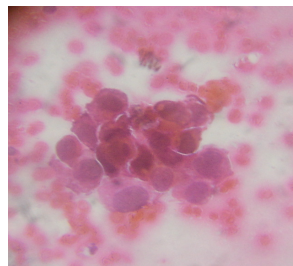


Fig-4: REAP-stained smear shows clumps of severe dysplastic cells(HP)

Discussion

The cervico-vaginal cytology is universally known as PAP test as it was popularised by George Papanicolaou. Today it is widely used both as a screening test in asymptomatic population & in the follow-up patients with cervical carcinoma. Following mass screening there has been reduction of 38% to 57% in the overall incidence of invasive carcinoma and a reduction of 67% in the incidence of clinically evident carcinoma. The original Papanicolaou stain of PAP smears had undergone various modifications [1, 4] in different laboratories. The Rapid Papanicolaou stain [2] is as fast as Diff-Quik and gives cytomorphological pictures as good as Papanicolaou stain but it requires a large quantity of expensive ethyl alcohol. In REAP, ethyl alcohol is replaced in most of the steps by 1% Acetic acid. Acetic acid which acts as mild dehydrating agent. It is cheap and easily available [5]. In this study, the cytoplasmic stain is optimal in 100 smears out of 110 (90%). The cyanophilia & eosinophilia of the cells (Fig.1 & 3) were comparable with Papanicolaou stain. Rest 10 smears show suboptimal stain because the stain could not penetrate the thick part. Nuclear staining requires demonstration of sharp nuclear features with crisp chromatin for diagnostic accuracy. In the present series, in 95% (105 out of 110 smears) cases nuclear staining (Fig.2 & 4) shows optimal features. Only in 5% occasions the nuclear staining is pale & suboptimal. The staining quality is preserved for the last 6 months. This is essential for smear review & retrospective studies. The cost factor plays the pivotal role in any organised screening programme, especially in developing country with high prevalence of the disease concerned. The REAP costs about 25% of total cost of standard Papanicolaou stain [5]; thereby it is cost-effective in mass screening programme. The method is also very rapid & suitable for the screening test.

Conclusion: REAP stain, in comparison to conventional Papanicolaou, provides a suitable, excellent & rapid alternative for cytological screening with minimum cost. Overall the use of absolute alcohol is minimum; it is used only during fixation and for dehydration before mounting. The stain preservation is also good in REAP method. It may be considered the suitable alternative to standard Papanicolaou stain for cervical cancer screening programme in our country with high prevalence of the disease.

Reference

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