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RESEARCH ARTICLE

Rapid Acetic Acid Papanicolaou Stain: An Economical and Rapid Substitute to conventional PAP Stain

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ABSTRACT

Background: Cervical cancer screening relies heavily on the Papanicolaou (PAP) stain, which, although effective, is time-consuming and relatively costly. In resource-limited and high-volume settings, there is a need for faster, economical, and efficient staining techniques such as the REAP method.

Aim: To evaluate and compare the staining quality of the Rapid Economical Acetic acid Papanicolaou (REAP) staining technique with Conventional PAP stain

Material & Method: We collected 372 cervical smears from 186 patients over the course of six month. Each patient provides two samples- one for routine examination for conventional PAP stain and the other for the REAP stain. One batch of smears was stained using the traditional PAP stain method, while the other was stained using the REAP technique which utilized 1% acetic acid instead of absolute alcohol in almost every step except during the fixation and just before mounting.

Result: The comparative analysis of the REAP stain versus the PAP stain highlights the REAP method as a feasible and effective alternative for cytological diagnosis, particularly in environments with limited resources and high sample volumes. REAP staining significantly reduces turnaround time, completing the process in approximately 3 to 6 minutes, as opposed to the 20 to 30 minutes required for the conventional PAP method.

Conclusion: REAP staining is faster, cheaper, and simpler than PAP, making it suitable for high-throughput and low-resource settings while maintaining strong diagnostic accuracy. Despite slightly less cytoplasmic detail, it shows excellent nuclear clarity, higher abnormal detection, and fewer unsatisfactory slides, making it ideal for large-scale screening.

Keywords: Conventional PAP Stain, REAP staining, PAP smear

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Introduction

Dr. George Nicholas Papanicolaou, a Greek-American pathologist introduced the papanicolaou (PAP) staining method in 1943.[1] The PAP stain is a widely utilized cytological technique for detecting inflammatory conditions and identifying cancerous lesions during screening procedures. Dr. Papanicolaou's initial modifications to the PAP staining technique, first developed in 1943, were later documented and published in the years 1954 and 1960. The Pap test became widely adopted for cervical cancer screening which has led to a significant decrease in cervical cancer-related deaths has been observed in countries where screening programs are widely implemented. Primarily used for cytological screening –especially in cervical cancer detection also used in other body fluid cytology like Sputum, Urine, and Effusions.[2]

As a polychromatic staining method, it employs a combination of dyes to distinguish between different cellular elements. This technique is particularly useful for assessing cell maturity, detecting nuclear irregularities, and identifying malignant cells.[3]

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In the Indian context, the conventional PAP staining method presents practical challenges, as laboratories are required to obtain a government-issued license to procure alcohol, which is often supplied in limited and inconsistent quantities. To address this issue, Dighe et al. [4] introduced a modification by substituting alcohol with 1% acetic acid in most steps of the procedure. 1% Acetic acid, being a mild dehydrating agent, is both cost-effective and readily accessible. This adapted technique has been named the Rapid, Economical, Acetic acid Papanicolaou (REAP) stain. The REAP method demonstrated optimal cytoplasmic clarity and differentiation in 90.5% of cases, while nuclear detail and chromatin definition were distinct and sharp in 96% of samples. A similar outcome was reported in a separate study conducted by Biswas et al. [5]

The modified Papanicolaou technique was developed with the aim of minimizing both the time and expense involved in the staining process, while maintaining the diagnostic quality and accuracy of the cytological smears. [6]

The conventional PAP stain is a 20 min procedure and uses a significant amount of absolute alcohol as the dehydrating agent, which is highly expensive and difficult to obtain in a developing country like India. [7]

Many authors have standardized and compared a variety of alterations in the conventional pap stain to decrease the turnaround time and to reduce the use of alcohol. Various modifications of pap stain such as Ultra-Fast and rapid pap have reduced the time to a minimum of 90 s, but the issues of ethanol and color preservation still remains. [8, 9]

Aim of the Study

The aim of this study is to evaluate and determine whether the Rapid Economical Acetic acid Papanicolaou (REAP) staining method could be a suitable and cost-effective alternative to the traditional Papanicolaou staining technique.

Material And Methods

This prospective cross-sectional study involved the collection of 372 cervical smears from 186 patients who visited the outpatient department (OPD) over the course of six month. Each patient provides two samples- one for routine examination for conventional PAP stain and the other for the REAP stain. Informed consent was obtained from every patient before collecting the sample.

The smears prepared were fixed using 95% ethyl alcohol. Two batches of the smears were made and labeled. One batch of smears was stained using the traditional PAP stain method, while the other was stained using the REAP technique which utilized 1% acetic acid instead of absolute alcohol in almost every step except during the fixation and just before mounting.

In the Conventional PAP stain procedure, the fixed smears were treated with a series of decreased concentration of ethyl alcohol solution, making 10 dips each, prior to the application of nuclear stains.

Staining components

- Hematoxylin – stains nuclei blue to dark purple, shows chromatin details clearly
- Orange G (OG-6) – stains Keratinized cells bright orange, useful for identifying squamous differentiation
- EA Stains (EA-36, EA-50, and EA-65) –stains cytoplasm of cells: Superficial cells –pink/red, intermediate cells: green/blue-green

The specific staining pattern depends on the cell type and its metabolic activity.

Calculation of sample size:

The sample size (n) is calculated according to the formula:
$n=z2 * p *(1 – p) / e2$
Where: z =1.96 for a confidence level (α) of 95%, p = proportion (expressed as a decimal), e = margin of error.
z = 1.96, p = 0.86, e = 0.05
n = 1.962 * 0.86 * (1 – 0.86) / 0.052
n =04625 / 0.0025 = 185.011
n = 186
The sample size is equal to 186

A comprehensive report, including the final diagnosis, was then generated based on their observations. Following this, the smears stained using the conventional method were revisited, and the staining quality, cytological features, and diagnostic outcomes of the REAP-stained slides were systematically compared to those obtained with the conventional PAP method.
Scoring System (for cytological quality): All smears were assessed and scored on scoring quality based on nuclear details, cytoplasmic details and background.
Each parameter was scored from 1-3:
Score 1 - Poor
Score 2 -Moderate
Score 3- Good
In addition to these comparisons, an analysis was conducted to assess the cost-efficiency and reduced processing time offered by the REAP technique relative to the traditional staining approach.

Table 1: Procedure for Conventional PAP and REAP staining [5]

Conventional PAP stain		*REAP*	
95% alcohol (fixation)		95% alcohol (fixation)	10 dips
95% alcohol	10 dips	1% acetic acid	
80% alcohol	10 dips	-	
70% alcohol	10 dips	-	
50% alcohol	10 dips	-	
Tap water	10 dips	-	
Harris haematoxylin	2 min	Harris haematoxylin, preheated 60◦C	10 dips
Scott's Tap water	3-5 min	Tap water	10 dips
50% alcohol	10 dips	-	
70% alcohol	10 dips	-	
80% alcohol	10 dips	1% acetic acid	10 dips
95% alcohol	10 dips	-	
OG -6	1 min	OG-6	10 dips
95% alcohol	10 dips	-	
95% alcohol	10 dips	1% acetic acid	10 dips
EA	10 min	EA	10 dips
95% alcohol	40 dips	1% acetic acid	10 dips
95% alcohol	40 dips	-	
100% alcohol	10 dips	Methanol	10 dips
Xylene	10 dips	Xylene	10 dips
Xylene	10 dips	-	
Xylene	10 dips	-	
DPX mount	Coverslip	DPX mount	Coverslip

Result

The age of the patients included in the study varied between 21 and 60 years, with a mean age of 40.5 years.

The chart illustrates the distribution of 186 samples across four age groups. Aged between 31-41yrs has the highest representation, with approximately 102 samples (55%), indicating its dominant presence. 21-31yrs follows with around 47 samples (25%), showing a moderate share. 41-51yrs accounts for about 28 samples (15%), while 51-61yrs is the least represented, only 9 samples (5%). This distribution suggests that 31-41yrs age is most significant in the datasheet.

Optimal staining is higher with REAP for both cytoplasmic (184 vs. 182) and nuclear (183 vs. 180) features. Suboptimal staining is lower with REAP for both cytoplasmic (2 vs. 4) and nuclear (3 vs. 6) features.

Table 2: Age group distribution

Age	*Number*	*Percentage*
21-31 YRS	47	25%
31-41 YRS	102	55%
41-51 YRS	28	15%
51-61 YRS	9	05%

The Conventional PAP stain demonstrates higher mean scores across all evaluated parameters compared to the REAP method, indicating slightly better staining quality overall in this scoring system. The PAP stain consistently outperforms REAP in all three staining quality parameters — nuclear, cytoplasmic, and background clarity.

Table 3: Result of staining quality by conventional PAP stain and REAP stain

Procedure	*Optimal*		*Suboptimal*	
	Cytoplasmic	*Nuclear*	*Cytoplasmic*	*Nuclear*
Conventional PAP stain	182	180	04	06
REAP stain	184	183	02	03

Table 4: Comparison of Staining Quality Scores

Stain Type	*Nuclear Detail (Mean ± SD)*	*Cytoplasmic Detail (Mean ± SD)*	*Background (Mean ± SD)*
PAP	2.9 ± 0.2	2.8 ± 0.3	2.7 ± 0.4
REAP	2.7 ± 0.3	2.6 ± 0.4	2.5 ± 0.4

Although differences are small (0.2 points on average) and within a "Good to Moderate" quality range, they may become significant in borderline or diagnostically challenging cases.

The slightly lower scores with REAP may be acceptable in routine screening, especially when balanced against faster turnaround time and lower cost

All cytological smears were categorized based on the Bethesda system for cervical cytology into the specified diagnostic groups, and these classifications were subsequently compared using the conventional PAP stain as the reference standard.

REAP showed 2 more cases in NILM and ASC-US category. While PAP stain showed 1 more case in inflammatory category, 3 more cases in LSIL category. However, they showed similar findings in HSIL category. REAP showed similar diagnostic work up. There was no significant disparity observed between REAP and PAP stain results.

Turnaround Time: REAP reduces the average staining time from 25 minutes to just 8 minutes—a 68% reduction in processing time. This substantial time saving enhances workflow efficiency, enabling quicker reporting and higher daily sample capacity, especially in busy or high-volume laboratories.

Table 5: Diagnostic Concordance with Conventional PAP Stain

Diagnosis	*PAP Stain (Gold Std)*	*REAP(Agree)*
NILM	86	88
Inflammatory	42	41
ASC-US	28	30
LSIL	21	18
HSIL	9	9
Total	186	186

Discussion

For over five decades, the Papanicolaou stain, originally developed and later refined by Dr. George N. Papanicolaou, has served as the cornerstone of cervical cancer screening programs worldwide. [1] Renowned for its diagnostic reliability, this staining technique has become a standard tool in cytopathology. Throughout the years, numerous laboratories and researchers have explored modifications to the traditional protocol in an effort to improve efficiency and adaptability. The quest has primarily focused on creating a faster staining method comparable in speed to rapid stains like Diff-Quik without compromising the superior cytomorphological details that the original PAP stain is known for. These efforts have led to various adaptations of the conventional method, each aiming to maintain diagnostic accuracy while enhancing turnaround time, particularly in high-throughput or resource-limited settings. [10]

By assessing these key parameters, the study sought to determine whether REAP could serve as a practical substitute for routine use in mass screening initiatives. Staining quality was assessed by examining the characteristics of cytoplasmic and nuclear staining.

The comparative analysis of the REAP stain versus the PAP stain highlights the REAP method as a feasible and effective alternative for cytological diagnosis, particularly in environments with limited resources and high sample volumes. REAP staining significantly reduces turnaround

Table 6: List of parameters comparing REAP and PAP stain

Parameters	*Reap Stain*	*Pap Stain*
Number of samples	186	186
Staining Time	3-6 minutes	20-30 minutes
Cost per slides	Lower	Higher
Clarity of cytoplasmic details	Good	Excellent
Nuclear Details	Excellent	Excellent
Background Cleanliness	Clean	Clean
Detection of Abnormal cells	95%	92%
Unsatisfactory slides (%)	2%	6%
Suitable for Mass Screening	Excellent	Moderate
Reproducibility	High	High
Slide Interpretation Time	Shorter	Longer
Staining Complexity	Simple	More Complex

Table 7: List of parameters comparing REAP and PAP stain

Parameters	*Reap stain*	*Pap stain*
Number of samples	186	186
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Cost per slides	Lower	Higher
Clarity of cytoplasmic details	Good	Excellent
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Background Cleanliness	Clean	Clean
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Unsatisfactory slides (%)	2%	6%
Suitable for Mass Screening	Excellent	Moderate
Reproducibility	High	High
Slide Interpretation Time	Shorter	Longer
Staining Complexity	Simple	More Complex

time, completing the process in approximately 3 to 6 minutes, as opposed to the 20 to 30 minutes required for the conventional PAP method.[11]

Diagnostic performance is promising; with REAP achieving a slightly higher abnormal cell detection rate (95%) compared to PAP (92%), and a lower percentage of unsatisfactory slides (2% vs. 6%), indicating greater reliability and consistency in sample evaluation. Both methods exhibit excellent nuclear detail and clean background staining, while REAP provides good, though slightly less superior, cytoplasmic detail than conventional PAP.

- Operational Efficiency: Quicker staining, simpler protocol, faster interpretation.
- Cost Savings: Lower per-slide cost makes it ideal for large-scale use.
- Scalability: Ideal for large-scale screening initiatives, particularly in resource-limited environments.

The present study reinforces the continued relevance of cytological staining innovations in improving cervical cancer screening efficiency without compromising diagnostic accuracy. While the conventional Papanicolaou stain remains the gold standard due to its superior cytoplasmic transparency and crisp nuclear detail, the REAP

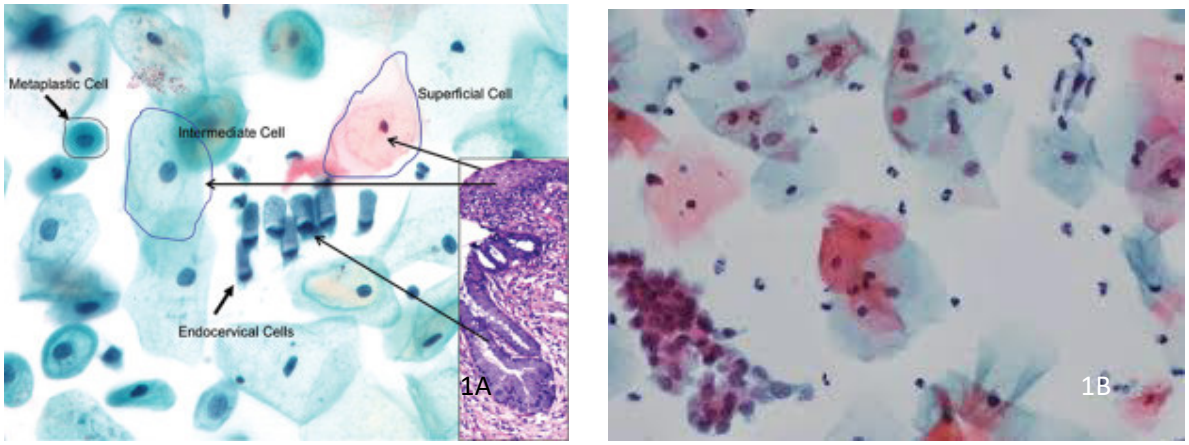

Figure 1: (A) - Conventional PAP stain. (B)- REAP staining method

method demonstrates that comparable diagnostic outcomes can be achieved with significantly reduced time and cost.[12]

The slightly lower cytoplasmic scores observed with REAP (mean 2.6 vs. 2.8 in PAP) can be attributed to the substitution of alcohol with 1% acetic acid. Alcohol acts as a stronger dehydrating agent and enhances cytoplasmic transparency, whereas acetic acid, though effective, produces comparatively softer staining. However, this minor reduction did not significantly interfere with diagnostic interpretation, as evidenced by the high concordance rates across all Bethesda categories.

Figure 1 A and 1B- Illustrates the polychromatic nature of PAP staining, where differential staining allows clear identification of cell maturity and origin. In the present study, REAP staining successfully replicated this differentiation pattern, though with slightly less intensity in cytoplasmic hues.

Clinical and Practical Significance

The reduced turnaround time (8 minutes vs. 25 minutes) is not merely a technical advantage—it has direct clinical implications:

- Faster reporting enables early diagnosis and intervention
- Increased daily sample processing improves screening coverage
- Reduced reliance on alcohol minimizes regulatory and supply constraints, especially in countries like India

Additionally, the lower percentage of unsatisfactory smears in REAP (2%) suggests improved sample usability, which is critical in large-scale screening programs where repeat sampling may not be feasible.

The findings of our study are consistent with previous studies by Dighe et al. and Biswas et al.,[4,5] which reported comparable nuclear detail between REAP and PAP with slight compromise in cytoplasmic staining and significant reduction in cost and time.[14]

However, our study strengthens the evidence by demonstrating higher abnormal cell detection (95%), suggesting that REAP may even enhance sensitivity in certain cases.

Despite its advantages, REAP staining has certain limitations:

Slightly reduced cytoplasmic transparency may affect interpretation in borderline cases

Long-term color preservation and slide archival quality need further evaluation

Multicentric studies with larger sample sizes are required to validate reproducibility

Future research could focus on integrating REAP with automated cytology screening systems and evaluating its role in HPV co-testing protocols.

Overall, the findings of this study suggest that the REAP staining method effectively balances diagnostic reliability, operational efficiency, and cost-effectiveness, making it a highly practical alternative to conventional PAP staining, particularly in high-volume and resource-constrained healthcare settings.

Conclusion

In summary, REAP staining technique demonstrates significant advantages over the conventional PAP stain, especially in key areas relevant to high-throughput and resource-limited cytology laboratories. Its significantly shorter staining time, lower cost per slide, and simplified procedure enhance laboratory efficiency and make it more accessible for widespread use, especially in mass screening

and rural healthcare settings. Despite offering slightly less cytoplasmic detail, REAP maintains excellent nuclear clarity and diagnostic accuracy, with a higher abnormal cell detection rate and fewer unsatisfactory slides compared to the PAP method. Therefore, the REAP method not only maintains diagnostic reliability but also addresses key logistical and economic barriers, making it a more effective and sustainable choice for large-scale cervical cancer screening programs and general cytopathological diagnostics.

References

1. Izhar S, Kaur R, Masih K. Efficacy of rapid, economical, acetic acid, Papanicolaou stain in cervical smears as an alternative to conventional Papanicolaou stain J Cytol. 2014;31:154–7
2. Gill GW. EnviroPap: An environmentally friendly, economical, and effective Pap stain. Lab Med Indianapolis 2006; 37:1058.
3. Krishnan S, Madsen E, Porterfield D, Varghese B. Advancing cervical cancer prevention in India: Implementation science priorities. Oncologist 2013; 18(Suppl):1325
4. Dighe SB, Ajit D, Pathuthara S, and Chinoy R. Papanicolaou stain: Is it economical to switch to rapid, economical, acetic acid, and Papanicolaou stain? Acta Cytol 2006; 50:643-6.
5. Biswas RR, Paral CC, Dey R, Biswas SC. Rapid, economic, acetic acid, Papanicolaou stain (REAP) is it suitable alternative to standard PAP stain? Al Ameen J Med Sci 2008; 1:99-103.
6. Gachie RN, Muchiri LW, Ndungua JR. A Comparison of modified and standard papanicolaou staining methods in the assessment of cervical smears at Kenyatta national hospital. East Afr Med J 2011; 88:24450.
7. Prasad PR. Shortduration Papanicolaou stains (SPS)—an alternative to conventional Papanicolaou stain in routine cytopathology? Comp Clin Pathol 2017; 26:12858.
8. Shree VR, Das S. Comparative evaluation of conventional Papanicolaou stain (PAP) stain efficacy versus modified ultrafast Papanicolaou stain (MUFP) stain Arch Cytol Histopathol Res. 2019;4:116–7
9. Gupta S, Chachra KL, Bhadola P, Sodhan P. Modified Papanicolaou staining protocol with minimum alcohol use: A cost-cutting measure for resource-limited settings Cytopathology. 2010;21:229–33
10. Agarwal P, Toi PC, Subramanian H, Apoorva Lakshmi S. Prospective comparison of cytological specimen adequacy assessment by different rapid staining techniques for rapid onsite evaluation in fine needle aspiration cytology and their costeffectiveness. Diagn Cytopathology 2019; 47:46974.
11. *Pak HY, Yokota SB, Teplitz RL. Rapid staining techniques employed in fine needle aspirations. Acta Cytol. 1983;27:81–3.*[PubMed] [Google Scholar]→ Essential reference for your diagnostic categorization (NILM, ASC-US, LSIL, HSIL).
12. Choudhary P, Sudhamani S, Pandit A, Kiri V. Comparison of modified ultrafast Papanicolaou stain with the standard rapid Papanicolaou stain in cytology of various organs. J Cytol 2012;29:2415.
13. Holowaty P, Miller AB, Rohan T, To T. Natural history of dysplasia of the uterine cervix. J Natl Cancer Inst 1999;91:2528.
14. Singh VB, Gupta N, Nijhawan R, et al. *A comparison of conventional Pap smear and liquid-based cytology*. J Cytol. 2012;29(2):86–89.→ Useful for broader comparison of cytological techniques.