

Assessing the Diagnostic Utility of REAP Stain in Cervical Cytology: An Economical Alternative for Rural Healthcare Practice**Preeti Mishra¹, Samarth Shukla², Keshav Yadav³, Neema Acharya⁴, Sunita Vagha⁵, Suhit Naseri⁶**¹Assistant Professor, Department of Pathology, Jawaharlal Nehru Medical College, DMIHER, Wardha, Maharashtra, India.²Professor, Department of Pathology, Jawaharlal Nehru Medical College, DMIHER, Wardha, Maharashtra, India.³Junior Resident, Department of Pathology, Jawaharlal Nehru Medical College, DMIHER, Wardha, Maharashtra, India.⁴Professor and Head, Department of Obstetrics and Gynaecology, Jawaharlal Nehru Medical College, DMIHER, Wardha, Maharashtra, India.⁵Professor and Head, Department of Pathology, Jawaharlal Nehru Medical College, DMIHER, Wardha, Maharashtra, India.⁶Senior Resident, Department of Pathology, Datta Meghe Medical College, DMIHER, Nagpur, Maharashtra, India**Received: 20-06-2026 / Revised: 19-07-2026 / Accepted: 22-08-2026****Corresponding Author: Dr. Preeti Mishra****Conflict of interest: Nil****Abstract:**

Cervical Cancer remains a global health threat. There are many countries where there is a lack of resources and funding to utilize the available screening methods. The Pap Smear, also known as the Papanicolaou Test, utilizes Cytology to screen for abnormal cells on the cervix and vagina. The traditional Pap Stain is the "Gold Standard" for viewing abnormalities because of the detail in both nuclei and cytoplasm. Although the traditional Pap stain provides optimal detail, it is slow, expensive and requires the use of Alcohol, making it difficult to perform in areas of limited resources.

The Rapid Economical Acetic Acid Papanicolaou (REAP) Stain has emerged as a viable alternative to the traditional Pap Stain. The REAP Stain can be performed quickly, at a lower cost than the traditional Pap stain and does not require the use of Alcohol but instead uses Acetic Acid. The purpose of this study was to evaluate the effectiveness of the REAP Staining Technique used in cervicovaginal cytology and compare it to the Traditional Pap Stain based on Quality of Staining, Time to Complete Staining, Ease of Interpretation, Cytoplasmic Detail, Nuclear Detail and Chromatin Clarity. Two hundred cervical smears were collected and each sample had two slides created for both the traditional Pap Stain and the REAP Stain. Each slide was then reviewed based upon cytoplasmic staining, nuclear detail, chromatin clarity and overall ease of reporting. Based upon the results of the study, it appears that the REAP Stain offers a number of benefits including quicker staining times and less expense than the traditional Pap stain. In contrast, it offered inferior nuclear detail and chromatin clarity when compared to the traditional Pap stain. Based upon these findings, the REAP Stain could be utilized for mass screening purposes in underdeveloped or resource poor environments.

Keywords: Cervical cancer, Pap smear, REAP stain, Cervicovaginal cytology, Cytological screening, Papanicolaou stain, Cost-effective staining, Rapid staining techniques.

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Introduction

There is a continued need for action against cervical cancer that is considered a major global health issue. Cervical cancer is also one of the primary sources of cancer-related morbidity and mortality among women globally, especially within lower- and middle-income countries [1].

This disparity is primarily due to the limited availability of organized screening programs, low levels of awareness about cervical cancer prevention and treatment options, and limited access to healthcare services. Even though there is a considerable amount of time before cervical cancer develops into an invasive type of cancer during the preinvasive phase of the disease, providing ample

opportunities for early detection and treatment of cervical cancer, many women are still being diagnosed at late stages. Therefore, this contributes to poor clinical outcomes for patients who receive delayed diagnosis [2].

Screening for cervical cancer represents a highly effective strategy for reducing the burden associated with this disease. There are a number of different screening methods that can be utilized to screen for cervical cancer, however cytology-based screening utilizing the Papanicolaou (Pap) smear has proven to be particularly useful in identifying individuals with abnormal cell changes or early invasive carcinoma. The implementation of Pap smear screening programs within higher-income countries has contributed to substantial declines in both incidence and mortality rates of cervical cancer [3].

Additionally, The Bethesda System for Reporting Cervical Cytology has provided a standardized approach to reporting cytologic interpretations. This system has increased the consistency and reliability of cytologic diagnoses by allowing clinicians to make more informed decisions. The success of cervical cytology is dependent upon the quality of staining; the quality of staining affects how well cellular characteristics such as nuclei and cytoplasm are visualized. The original Papanicolaou staining technique was introduced in the early 20th Century. The Papanicolaou stain is currently referred to as the gold standard because of its ability to produce polychromatic, clear staining with superior nuclear detail and cytoplasmic differentiation. These features are required for accurate identification of epithelial alterations including dysplasia and malignancies. However, despite the fact that the Papanicolaou stain produces superior diagnostic results, the traditional Pap stain contains a number of practical disadvantages. The process is time consuming, multi-step and utilizes large amounts of alcohol. The use of large amounts of alcohol will increase the cost of performing Pap stains and limit their use in resource constrained environments [4].

Therefore, various modifications of the Papanicolaou staining method were developed with the intention of shortening the duration required to complete the staining process, decreasing the complexity of the process and decreasing costs while maintaining acceptable levels of diagnostic accuracy. Some examples of modifications to the Pap staining method include rapid Pap stain, ultra-fast Pap stain and alcohol-free staining processes. Among these modifications, the Rapid Economical Acetic Acid Papanicolaou (REAP) stain has gained popularity as a possible alternative. Acetic acid replaces alcohol in the REAP staining technique.

The REAP staining technique reportedly is quicker than, less expensive than and simpler to utilize than

the traditional Pap stain. This could contribute to improved efficiency in screening activities, specifically in peripheral and rural healthcare facilities where laboratory equipment is limited. However, questions remain about the quality of the nuclear staining, chromatin clarity, and general cytomorphological detail produced by the REAP stain compared to the traditional Pap stain. Because accurate interpretation of cervical cytology is heavily reliant upon nuclear details, any loss in staining quality would negatively impact diagnostic accuracy.

For these reasons, it is important to conduct a systematic evaluation comparing the performance of the REAP staining technique with that of the traditional Pap stain. An assessment of cytoplasmic and nuclear details, staining quality and ease of interpretation is needed to establish if REAP can be utilized as a viable alternative for routine cytology procedures in environments where time and cost are critical limitations.

Aim of the Study: To evaluate the efficiency of a new rapid economical acetic acid Papanicolaou (reap) staining process in cervicovaginal cytology and to compare it to traditional Papanicolaou (pap) stain on the basis of cytomorphologic quality, staining attributes, staining time, cost-effectiveness and general diagnostic applicability, in order to ascertain whether it is suitable as an alternative method for cervical cancer screening. This would be especially valuable in resource-poor settings.

Objectives

- 1.) To evaluate the qualitative features of nuclei and cytoplasm of smear preparations stained using the REAP method.
- 2.) To compare the qualitative attributes of the cytoplasm and nuclei of REAP stained smears and those conventionally prepared and stained with Pap stain, including the time required for staining.
- 3.) To determine if there are advantages to reporting cervicovaginal cytology from REAP-stained smears.

Material and Methods

This study was conducted in Division of cytology in Department of Pathology JNMC Sawangi (Meghe), Wardha. In duration of one year from october-18 to october-19.

Study Design and Setting: Cervicovaginal cytology is widely used as a primary screening modality for early detection of premalignant and malignant lesions of the cervix; therefore, standardized techniques of cytological diagnosis are essential for reliable diagnosis [5,6].

Study Duration: The study was carried out over a period of one year.

Study Population: A total of 200 patients visiting the outpatient clinic at the Gynecology Department participated in this study. Patients with cervicovaginal cytologic examination had been referred to the Cytology Department by clinicians for either routine screening or for assessment of their symptoms, e.g., vaginal discharge, menstrual disorders or abnormal bleeding from vagina. Routine screening in such patient groups allows for early recognition of the cervical epithelial alterations and improve treatment results.

Inclusion Criteria

- Patients with symptoms including vaginal discharge, menstrual cycle irregularities and anemia due to abnormal uterine bleeding.
- Patients participating in a routine cervical-vaginal smear (cytology) screening program.

$$N = \frac{\chi^2 * N * p * (1-p)}{C^2 * (N-1) + \chi^2 * p * (1-p)}$$

$$= 196.16$$

= 200 patients needed in the study

The calculated sample size was about 196. The sample size was rounded to 200 to ensure that the results would be statistically valid [8].

Ethical Considerations: The research in this study was completed under the guidelines of an institution's ethics committee. There were no major ethical concerns because the process included standard clinical cytological examination that did not require any additional invasive procedures. All patients provided informed consent for the samples collected as well as their personal information remained confidential throughout the process [9].

Sample Collection Procedure: Cervical smears were taken on outpatients with a general practitioner at the gynecology outpatient clinic. Two smears per patient are prepared. Samples are gathered by scraping the transformation zone of the cervix with either an Ayres spatula or cytobrush to ensure sufficient cellular material. Accurate methods of collecting the smear is essential for obtaining representative squamous and glandular cells and also to avoid potential sampling errors. Preparing the smears as soon as possible after the sampling process will prevent excessive drying due to air exposure and maintain morphological characteristics of cells [10].

Fixation: Immediate fixation of at least one smear per patient with a fixative such as 95% Ethanol is recommended to preserve nuclear details, chromatin patterns and cytoplasmic detail [11]. The other smear was processed utilizing the REAP staining method that uses Acetic Acid as opposed to Alcohol, therefore the smears do not have to be fixed conventionally.

- Patients able to give informed consent for this research.

Exclusion Criteria

- Patients whose smears are likely to be too poorly cellularized e.g. those over 60 years of age.
- Smears that have been poorly preserved or inadequately sampled.
- Patients who do not wish to participate in this research.

Sample Size

The sample size was calculated using standard statistical formula:

Total 200 cases were included in the present study. The sample size was calculated.

Staining Techniques

Conventional Papanicolaou (Pap) Stain: The traditional Pap staining method follows a typical laboratory staining process. There are five stages in this process. These include: fixation, hematoxylin-based nuclear staining, differentiation, hematoxylin blueing, cytoplasmic staining with Orange G solution, dehydration, clearing and finally, mounting. This is a multicolored staining that has excellent nuclear detail, along with good cytoplasmic clarity which makes this method the "gold standard" for cervical cytologic evaluation.

Rapid Economical Acetic Acid Papanicolaou (REAP) Stain: The REAP staining method utilizes acetic acid instead of alcohol in its papain-based staining process to reduce costs and shorten the staining time and therefore can be employed for high volume or resource limited laboratories.

The total staining time using the REAP method is estimated at 5-7 minutes, however, it was noted that the quality of the cytoplasmic staining with the REAP method was acceptable but there were some differences in nuclear detail and chromatin clarity compared to the traditional pap stain [13,14].

Blinding and Slide Coding: To prevent observer bias from occurring with respect to the smears, all smears were coded and labeled with an independent identifier; therefore, the patient's original ID was removed from the slide and both pathologists that independently evaluated the smears had no knowledge of which stains were used. By having the pathologists evaluate the smears without prior knowledge of the stains used, it would enhance their objectivity when evaluating these smears, reduce

inter-observer variability in cytology interpretations and provide consistent results throughout this study [15].

Evaluation Parameters: All smears were evaluated through light microscopy, and then each smear was evaluated for cytomorphologic characteristics using defined criteria. These specific criteria included:

- The borders of cytoplasm.
- The nuclear membrane of each cell.
- The chromatin pattern observed within each nucleus.
- The overall quality of the staining of each smear.

In order to evaluate each criterion listed above, a modified scoring method was employed and each of the criteria identified above was assigned scores of either 0–1, denoting "suboptimal", or 2–3, denoting "optimal" and previously referenced studies [13].

Results

A total of 200 cervicovaginal smears were included in the study. Each case was evaluated using both conventional Papanicolaou (Pap) stain and Rapid Economical Acetic Acid Papanicolaou (REAP) stain.

Age Distribution: The age distribution of patients included in the study is shown below:

Table 1: Age Distribution (n = 200)

Age Group (years)	Number of Cases	Percentage (%)
25–35	74	37%
36–45	41	20.5%
46–55	55	27.5%
56–65	30	15%

The majority of patients belonged to the 25–35 years age group (37%), followed by 46–55 years (27.5%). This indicates that most of the screened population was within the reproductive and perimenopausal age groups.

Comparative Evaluation of Cytomorphological Features: The cytological features of smears stained by conventional Pap stain and REAP stain were compared based on cytoplasmic borders, cytoplasmic staining, nuclear borders and chromatin staining.

Table 2: Comparison of Cellular Details between REAP and Conventional Pap Stain

Parameter	Feature	Score	REAP (n=200)	Conventional Pap (n=200)	χ^2 value	p-value
Cytoplasmic borders	Distinct	3	141 (70.5%)	198 (99%)	62.85	0.0001
	Indistinct	1	59 (29.5%)	2 (1%)		
Cytoplasmic staining	Satisfactory	3	135 (67.5%)	200 (100%)	77.61	0.0001
	Unsatisfactory	1	65 (32.5%)	0 (0%)		
Nuclear borders	Distinct	3	106 (53%)	200 (100%)	122.9	0.0001
	Indistinct	1	94 (47%)	0 (0%)		
Chromatin staining	Distinct	3	83 (41.5%)	181 (90.5%)	107	0.0001
	Hazy	1	117 (58.5%)	19 (9.5%)		

Interpretation of Findings

Cytoplasmic Borders: Distinct cytoplasmic borders were identified on 70.5 percent of specimens stained with REAP, versus 99 percent stained using the conventional Papanicolaou method. Borders that were indistinguishable from one another were found at a much greater rate within REAP (29.5 percent) than in conventional Papanicolaou (1 percent), which was statistically different ($p=0.0001$).

Cytoplasmic Staining: The quality of cytoplasmic staining as judged by both methods was also evaluated. Cytoplasmic staining of sufficient quality was present in 67.5 percent of REAP specimens while it was present in every specimen (100 percent) that was stained using the traditional Papanicolaou method. In addition, insufficient cytoplasmic

staining for evaluation was seen in 32.5 percent of REAP specimens. The difference was statistically significant ($p=0.0001$).

Nuclear Borders: More than half (53%) or just a little over half, of REAP smears showed distinct nuclear borders as opposed to 100%, or completely, of conventional Pap smears. More than a third (47%) of the REAP smears had unclear or indistinct nuclear borders, showing less clarity than those from conventional Pap smears. The difference is extremely statistically significant ($p = .0001$).

Chromatin Staining: A similar trend was observed with respect to chromatin patterns. Only about forty-one percent (41.5%) or slightly more than one-third of the REAP smears showed a clear chromatin pattern, while approximately ninety percent (90.5%)

of conventional Pap smears did so. A much larger percentage of the REAP smears (about fifty-nine percent; 58.5%) presented hazy chromatin versus about ten percent (10%) of the conventional Pap smears (9.5%). The difference is also statistically significant ($p = .0001$).

Overall Findings: The results clearly indicate that the conventional Pap smear provided better performance on each of the cytomorphologic parameters studied, specifically, cytoplasmic clarity, nuclear detail and chromatin definition.

Although the REAP stain appears to be faster and cheaper than the conventional Pap stain, it has notably lower staining quality relative to nuclear details important to accurate cytological diagnosis.

In addition to the obvious clinical implications for using an optimal stain technique when evaluating cervical cytology samples, statistical analysis revealed that all observed differences between the REAP and conventional Pap stains were highly statistically significant ($p < .05$). Thus, based on these findings, it can be concluded that the traditional Pap stain continues to represent the most dependable choice for obtaining detailed cytomorphologic information.

Discussion

Overview of Cervical Cytology in Screening: Cervical cancer remains a public health problem in many parts of the world especially developing nations where screening programs are not well organized. Cytology-based screening using Pap smears is useful for detecting premalignant lesions at an early stage and therefore reduces morbidity and mortality [12,13]. The effectiveness of these screening programs depends on both the quality of staining and the accuracy with which cytomorphologic features are interpreted [14].

Role of Conventional Pap Stain: Although it is considered the "gold standard" for cervical cytological diagnosis because of the quality of both nuclear and cytoplasmic details seen with the conventional Papanicolaou stain; there are a number of limitations to this method of staining cells such as: longer staining times than other methods require two or three different chemical stains to produce a quality cell smear that can be evaluated, and requires alcohol, which makes it difficult and expensive to utilize in low-resource countries [16].

Advantages of REAP Stain: The Rapid Economical Acetic Acid Papanicolaou (REAP) Stain was created as a solution to some of the disadvantages associated with the traditional method of staining cells. The use of acetic acid for replacing the alcohol in the REAP method is an economical way of reducing the amount of money required to

process samples; this also makes it a quicker process than many other methods that are used.

As such, the REAP method has several advantages over the traditional method when it comes to cost and speed which are both critical factors when conducting large numbers of tests and screenings in areas where resources may be limited [17].

Comparison of Cytoplasmic Features: Cytoplasmic bordering was clearly seen in much larger proportions of Pap-stained smears than of REAP stained smears. Specifically, approximately 99 percent of Pap-smears provided good cytoplasmic staining, as did all the Pap-smears examined, however only 70.5 percent of the REAP stains gave adequate cytoplasmic staining and only 67.5 percent of the REAP smears exhibited optimal cytoplasmic staining.

Therefore, although REAP may provide good enough cytoplasmic detail, no prior study has demonstrated that REAP is capable of providing clear and consistent cytoplasmic staining similar to that obtained using the conventional Pap staining technique. Other researchers have made similar observations when they evaluated various alternative staining procedures for cytologic screening [18].

Comparison of Nuclear Features: The best feature for diagnosing cytology is nuclear, as it forms the base of defining both dysplasia and malignancy. Based on this study, Pap staining provided better nuclear clarity than REAP, as 100 percent of smears had visible nuclear border lines and only 53 percent of smears stained by REAP were able to show a defined nuclear border line.

A significant number of smears (47 %) stained by REAP did not display clearly defined or recognizable nuclear borders lines which could potentially reduce the accuracy of diagnosis. This finding has been supported by previous studies demonstrating lessened definition within the nucleus when utilizing REAP staining methods [18,19].

Chromatin Pattern Evaluation: Distinctive chromatin was identified on a high percentage of Pap smear specimens taken from patients with conventional Pap (90.5%) as opposed to only 41.5% in the REAP smears; a significantly greater portion of REAP smears contained hazy chromatin (58.5%) than those from conventional Pap, therefore, there is potential to diminish diagnostic accuracy when attempting to identify subtle nuclear characteristics due to diminished resolution of the chromatin.

Studies conducted by Biswas et al. and Vani et al. also reported similar results. They concluded that while REAP stains can be used to screen cervical cells they are not capable of providing the detailed

nuclear information necessary for making a definitive diagnosis [19, 20]

Procedure	Cytoplasmic stain (score)		Nuclear stain (score)	
	Optimal (2-3)	Suboptimal (0-1)	Optimal (2-3)	Suboptimal (0-1)
Papanicolaou stain	3	1	3	1
REAP stain	2	0	2	0

Statistical Significance of Findings: The cytomorphologic characteristics examined in this study were found to be significantly different from one another by a p-value < .05 when comparing REAP and conventional Pap-stained samples. Therefore, these findings indicate that there is a clear advantage in both staining quality and diagnostic reliability associated with conventional Pap staining.

Clinical Implications: In addition to demonstrating how the REAP stain has advantages including faster staining times and less expense than conventional Pap staining methods; The REAP stain also demonstrated limitations in providing adequate nuclear detail and chromatin clarity.

Therefore, while the REAP stain may be an acceptable option for preliminary screening in large volume or resource poor settings, the traditional Pap stain should be used for confirmation and detailed cytological examination [17].

Correlation with Previous Studies: The current study's results were consistent with prior literature. Both studies by Biswas et al., and Vani et al. also found that although the REAP stain was less expensive and quicker than a traditional Pap stain, it lacked nuclear detail [19,20].

Consistent findings among different researchers provide further evidence for the traditional Pap stain being considered the "gold standard" while providing rationale for using the REAP stain in specific clinical situations.

Limitations of the Study

- Lack of histopathological correlation
- Subjective variation in cytological interpretation
- Limited evaluation of diagnostic sensitivity and specificity

Further large-scale studies incorporating histopathological correlation are recommended to validate these findings.

Future Perspectives: Future research should focus on refining rapid staining techniques to improve nuclear clarity while maintaining cost-effectiveness. Integration of such techniques with standardized reporting systems and quality control measures may enhance their applicability in large-scale screening programs.

Conclusion

It is essential that future studies develop and refine the development of rapid staining techniques which will increase the clarity of nuclear material while at the same time maintain low costs. A further refinement could be the use of rapid staining techniques in conjunction with standardized reporting methods and quality control processes in order to ensure widespread adoption of rapid staining techniques within the framework of large scale screening programs.

This study represents a comprehensive comparative analysis of the Rapid Economical Acetic Acid Papanicolaou (REAP) Stain and the traditional Papanicolaou (Pap) Stain used in cervicovaginal cytology. This study has demonstrated that the traditional Pap Staining Technique retains superior performance compared to the REAP Stain when evaluating cytomorphologic quality including nuclear clarity, chromatin detail and cytoplasmic transparency. These criteria are crucial in obtaining an accurate assessment of cytologic specimens, proper classification of epithelial cell abnormalities and providing an accurate cytologic diagnosis.

Although the REAP staining technique provides some practical benefits, including shorter staining times, lower cost and easier application than traditional Pap staining techniques, there exist several limitations associated with the REAP staining technique. Specifically, the REAP staining technique appears to have limited ability to visualize details within nuclei. Since morphology of cells and specifically morphology of the nucleus is the foundation upon which cytologic assessments are based, any loss in clarity of detail may decrease diagnostic certainty and accuracy. The increased prevalence of indistinct nuclear membranes and hazy chromatin observed using the REAP staining technique support this conclusion.

Although the above-mentioned limitations exist, the REAP stain does appear to provide adequate staining for cellular cytoplasm. As such, the REAP stain can be viewed as a viable alternative for certain clinical applications. The expedited turn-around time provided by the REAP stain along with lower cost of materials make it ideally suited for resource challenged areas, remote locations and high-volume screening programs where access and efficiency are primary concerns. Under these conditions, the use of

REAP stains may allow for greater geographic distribution of population-based screening activities and subsequently contribute to earlier detection of potential cancers.

However, it is necessary to note that the traditional Pap stain remains the "gold standard" for cervicovaginal cytology due to consistently better staining characteristics and diagnostic accuracy. Therefore, if possible, the traditional method should be utilized for definitive evaluations, especially when identifying subtle nuclear abnormalities.

Overall, the REAP stain may function as a complementary or initial screening tool under specific circumstances i.e., when utilizing the REAP stain in conjunction with the traditional Pap stain. Use of the REAP stain in cytologic practice should therefore be determined by clinical situation, availability of resources and need for diagnostic accuracy. Additional improvements in rapid staining techniques along with additional large studies involving histopathologic correlations may assist in bridging the gap currently present between cost effectiveness and diagnostic accuracy to ultimately increase the overall effectiveness of cervical cancer screening programs.

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This study involving data of human participants was conducted in accordance with the ethical principles of the Declaration of Helsinki, and informed consent was obtained from all participants.

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