

Comparison Of 96% Ethanol And 10% Formal Alcohol As Fixatives On The Staining Quality Of Papanicolaou-Stained Pleural Effusion Smears

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ABSTRACT

Background: Adequate fixation is an essential pre-analytical step in pleural effusion cytology because it preserves cellular morphology and supports optimal Papanicolaou staining. Although 96% ethanol is commonly used as a wet fixative, 10% formal alcohol may provide an alternative when conventional alcohol fixation is less practical. However, comparative information regarding the staining quality of pleural effusion smears fixed with these two solutions remains limited. **Objective:** This study aimed to compare the staining quality of Papanicolaou-stained pleural effusion smears fixed with 96% ethanol and 10% formal alcohol. **Methods:** A descriptive comparative laboratory study was conducted using 20 pleural effusion specimens. The specimens were divided into two fixation groups: 96% ethanol (n=10) and 10% formal alcohol (n=10). Cytological smears were prepared from pleural effusion sediments, fixed according to the assigned fixation group, and stained using the conventional Papanicolaou method. Staining quality was evaluated microscopically based on three parameters: nuclear–cytoplasmic contrast, background cleanliness, and staining uniformity. Data were analyzed descriptively using frequencies and percentages. **Results:** All specimens in both fixation groups demonstrated good overall staining quality (100%). Nuclear–cytoplasmic contrast and staining uniformity were classified as good in all specimens in both groups (100%). Differences were observed in background cleanliness. Good background cleanliness was observed in 7 of 10 smears (70%) fixed with 96% ethanol and 5 of 10 smears (50%) fixed with 10% formal alcohol. **Conclusion:** Both 96% ethanol and 10% formal alcohol produced good overall staining quality in Papanicolaou-stained pleural effusion smears. The two fixatives showed comparable nuclear–cytoplasmic contrast and staining uniformity, whereas 96% ethanol produced a higher proportion of smears with good background cleanliness. Thus, 96% ethanol remains the preferred fixative when optimal background cleanliness is required, while 10% formal alcohol may be considered as an alternative for routine cytological preparation.

INTRODUCTION

Pleural effusion is a common clinical manifestation associated with various diseases, including infection, inflammatory disorders, heart failure, and malignancy. Cytological examination of pleural effusion is an important diagnostic procedure for evaluating cellular abnormalities and supporting the differentiation of benign and malignant conditions. As a minimally invasive and relatively rapid examination, pleural effusion cytology is commonly used as an initial investigation in patients with suspected pleural disease. The quality of cytological interpretation depends not only on specimen adequacy but also on appropriate specimen processing and staining procedures (1-3).

Fixation is one of the most important pre-analytical steps in cytological preparation. Proper fixation preserves cellular morphology, minimizes cellular degeneration and autolysis, and maintains nuclear and cytoplasmic details required for microscopic evaluation. Inadequate or delayed fixation may result in morphological distortion, suboptimal nuclear staining, cytoplasmic changes, and increased background artifacts. These conditions may interfere with microscopic visualization and potentially reduce the quality of cytological assessment. For conventional Papanicolaou staining, 96% ethanol is widely used as a wet fixative because of its ability to rapidly preserve nuclear morphology and cellular details. Ethanol acts primarily as a coagulative fixative by precipitating cellular proteins and facilitating rapid fixation of freshly prepared smears. Despite its established use, the availability, cost, and storage requirements of ethanol may become practical considerations in some laboratory settings (1,2,4–6).

10% formal alcohol represents another fixation option that combines the coagulative effect of ethanol with the protein cross-linking properties of formaldehyde. This combination may provide adequate preservation of cellular architecture while offering practical advantages for routine laboratory use. However, the effect of formal alcohol on the staining characteristics of pleural effusion cytology smears requires careful evaluation, particularly because fixation can influence nuclear–cytoplasmic contrast and the amount of material retained in the smear background (5,7–10).

Previous studies have investigated different fixatives and preservative solutions for cytological specimens. However, comparative evidence specifically evaluating 96% ethanol and 10% formal alcohol in conventional Papanicolaou-stained pleural effusion smears remains limited. In particular, information regarding nuclear–cytoplasmic contrast, background cleanliness, and staining uniformity is still needed to support practical selection of fixatives in cytology laboratories (8–12).

Therefore, this study aimed to compare the staining quality of Papanicolaou-stained pleural effusion smears fixed with 96% ethanol and 10% formal alcohol based on nuclear–cytoplasmic contrast, background cleanliness, and staining uniformity.

METHODS

Study Design

This study employed a descriptive comparative laboratory design to evaluate and compare the staining quality of Papanicolaou-stained pleural effusion smears fixed using 96% ethanol and 10% formal alcohol.

Samples

A total of 20 pleural effusion specimens were included in the study. The specimens were divided into two fixation groups, consisting of 10 specimens fixed with 96% ethanol and 10 specimens fixed with 10% formal alcohol. The specimens were obtained from residual material of routine diagnostic examinations and were processed at the Cytohistotechnology Laboratory, Department of Medical Laboratory Technology, Poltekkes Kemenkes Bengkulu.

The specimens included in the study met the predetermined inclusion criteria and were considered suitable for preparation of cytological smears. Specimens with apparent damage or inadequate material for cytological preparation were excluded.

Cytological Preparation and Fixation

Fresh pleural effusion specimens were centrifuged to obtain cellular sediment. Smears were prepared from the sediment on clean glass slides and immediately fixed according to the assigned fixation group. In the first group, prepared smears were fixed using 96% ethanol. In the second group, prepared smears were fixed using 10% formal alcohol. Following fixation, all smears were subjected to the same conventional Papanicolaou staining procedure to ensure that the fixation solution was the primary difference between the two groups (2,5).

Papanicolaou Staining

All prepared and fixed smears were stained using the conventional Papanicolaou staining protocol. The staining procedure was performed under the same laboratory conditions for both fixation groups. Following staining, the slides were examined microscopically to assess cytological staining quality.

Evaluation of Staining Quality

Staining quality was evaluated microscopically using three predetermined parameters:

1. Nuclear–cytoplasmic contrast: assessed based on the clarity and distinction of nuclear and cytoplasmic staining. A smear was classified as good when nuclear and cytoplasmic components were clearly distinguishable and adequately stained. It was classified as poor when nuclear or cytoplasmic staining was unclear or insufficiently differentiated.
2. Background cleanliness: assessed based on the presence of debris, nonspecific staining, and other background artifacts. A smear was classified as good when the background was relatively clean with minimal interfering material. It was classified as poor when considerable debris, nonspecific staining, or background artifacts interfered with microscopic visualization.
3. Staining uniformity: assessed based on the evenness of staining across the evaluable smear area. A smear was classified as good when staining was relatively uniform, whereas it was classified as poor when marked variation or uneven staining was observed.

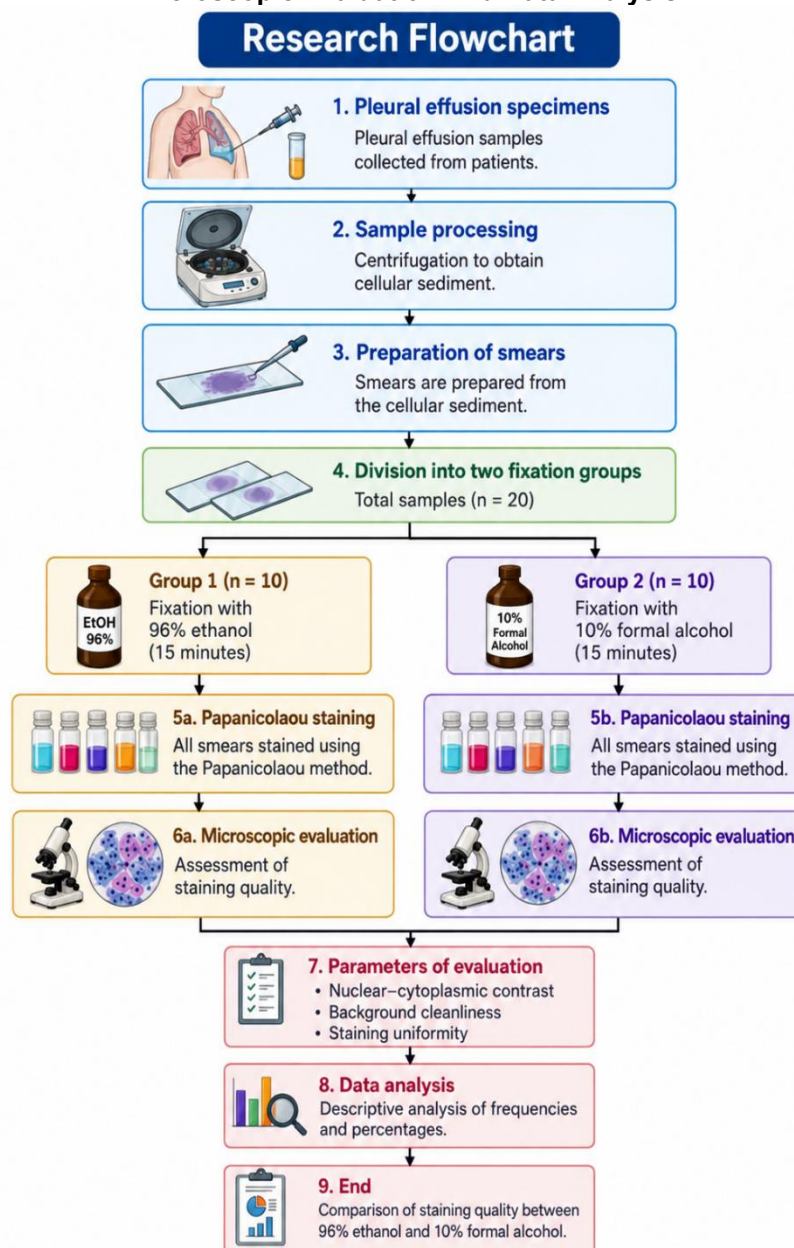
Each parameter was categorized as good or poor based on the predefined microscopic criteria. The overall staining quality was subsequently described according to the observed evaluation results.

Data Analysis

The data were analyzed descriptively and presented as frequencies and percentages for each evaluation parameter. The proportions of good and poor staining characteristics were compared descriptively between the 96% ethanol and 10% formal alcohol groups.

Because the study was designed as a descriptive comparative evaluation, the findings were interpreted primarily in terms of observed proportions rather than diagnostic accuracy or clinical performance.

Figure 1. The Flowchart Illustrates The Comparison Of 96% Ethanol And 10% Formal Alcohol As Fixatives For Papanicolaou-Stained Pleural Effusion Smears, From Specimen Collection To Microscopic Evaluation And Data Analysis



Source: Author's research, 2026.

Ethical Approval

This study received ethical approval from the Health Research Ethics Committee of Poltekkes Kemenkes Bengkulu (Approval No. KEPK.BKL/827/12/2025). Residual pleural effusion specimens obtained from routine diagnostic examinations were anonymized before analysis to maintain patient confidentiality.

RESULT

A total of 20 Papanicolaou-stained pleural effusion smears were evaluated, consisting of 10 smears fixed with 96% ethanol and 10 smears fixed with 10% formal alcohol.

Overall Staining Quality

Table 1. Overall Staining Quality of Pleural Effusion Smears According to Fixative

Fixative	Good n (%)	Poor n (%)
96% Ethanol	10 (100)	0 (0)
10% Formal Alcohol	10 (100)	0 (0)

Source: Author's research, 2026.

All smears fixed with either 96% ethanol or 10% formal alcohol demonstrated good overall staining quality. No smear in either group was classified as having poor overall staining quality.

Staining Quality According to Evaluation Parameters

Table 2. Staining Quality of Pleural Effusion Smears According to Evaluation Parameters and Fixative

Evaluation parameter	Fixative	Good n (%)	Poor n (%)
Nuclear–cytoplasmic contrast	96% Ethanol	10 (100)	0 (0)
	10% Formal Alcohol	10 (100)	0 (0)
Background cleanliness	96% Ethanol	7 (70)	3 (30)
	10% Formal Alcohol	5 (50)	5 (50)
Staining uniformity	96% Ethanol	10 (100)	0 (0)
	10% Formal Alcohol	10 (100)	0 (0)

Source: Author's research, 2026. Data are presented as frequency (n) and percentage (%).

Both fixation groups demonstrated comparable results for nuclear–cytoplasmic contrast and staining uniformity, with all specimens (100%) classified as good. Differences were observed in background cleanliness. The proportion of smears with good background cleanliness was higher in the 96% ethanol group (70%) than in the 10% formal alcohol group (50%). Representative photomicrographs are presented in Figure 2. Both fixation methods preserved the general cytomorphological appearance of pleural effusion cells and produced satisfactory Papanicolaou staining. Smears fixed with 96% ethanol generally demonstrated cleaner backgrounds, whereas smears fixed with 10% formal alcohol showed slightly more background material.

Figure 2. Representative Papanicolaou-Stained Pleural Effusion Cytology Smears Fixed With (A) 96% Ethanol And (B) 10% Formal Alcohol. Upper Panels Show Low-Magnification Views, While Lower Panels Show Higher-Magnification Views Demonstrating Cellular Morphology



Source: Researcher's documentation, 2026.

Overall, the two fixation methods produced comparable staining quality based on the evaluated parameters. The main observed difference was the proportion of smears with good background cleanliness, which was higher following fixation with 96% ethanol.

DISCUSSION

This study demonstrated that both 96% ethanol and 10% formal alcohol produced good overall staining quality in Papanicolaou-stained pleural effusion smears. All specimens in both fixation groups were classified as having good overall staining quality. Nuclear–cytoplasmic contrast and

staining uniformity were also classified as good in all specimens. However, the proportion of smears with good background cleanliness was higher in the 96% ethanol group than in the 10% formal alcohol group.

The comparable nuclear–cytoplasmic contrast observed in both groups suggests that both fixation solutions were capable of maintaining cellular structures that were adequately visualized after Papanicolaou staining. Nuclear preservation is particularly important in conventional Papanicolaou staining because nuclear chromatin, nuclear membrane characteristics, and cellular details contribute substantially to cytological assessment. The findings indicate that, under the conditions of this study, the use of 10% formal alcohol did not result in an apparent deterioration of nuclear–cytoplasmic contrast compared with 96% ethanol.

The staining uniformity was also classified as good in all specimens from both groups. This finding indicates that the two fixation methods did not produce an apparent difference in the evenness of staining across the evaluated smear areas. Consistent staining is influenced not only by fixation but also by subsequent staining procedures, including staining reagent conditions, timing, and processing consistency. Because the same Papanicolaou staining procedure was applied to both groups, the comparable results may indicate that both fixatives were compatible with the staining protocol used in this study.

A difference was observed in background cleanliness. Good background cleanliness was found in 70% of smears fixed with 96% ethanol compared with 50% of smears fixed with 10% formal alcohol. This observation suggests that 96% ethanol produced a cleaner microscopic background under the conditions evaluated. The difference in background characteristics may be related to the different fixation mechanisms of the two solutions. Ethanol acts primarily as a coagulative fixative, rapidly denaturing and precipitating cellular proteins. Rapid fixation immediately after smear preparation helps minimize cellular degeneration and may reduce the release of intracellular material into the smear background. This mechanism may contribute to the relatively cleaner background observed in the ethanol group (1,2,5).

In contrast, formal alcohol combines the coagulative effect of ethanol with the cross-linking effect of formaldehyde. Formaldehyde stabilizes cellular proteins through cross-linking, thereby supporting preservation of cellular architecture. However, this fixation mechanism may also contribute to the retention of proteinaceous material or other extracellular components within the smear. Such retained material may contribute to the slightly higher frequency of less-clean backgrounds observed in the 10% formal alcohol group (8,10).

The present findings are consistent with previous reports indicating that alcohol-based fixation provides satisfactory preservation of cytomorphological characteristics in conventional cytology. Sulochana et al. reported effective fixation of body fluid cytological specimens using alcohol-based fixatives, while Shabeeba and Geetha demonstrated satisfactory cellular preservation and staining characteristics with ethanol fixation. These findings support the established role of ethanol as a reference fixative for conventional cytological smears (6,9).

Previous research has also demonstrated that formaldehyde-containing fixation methods can provide satisfactory preservation of cellular morphology. Ireka et al. reported preservation of cytological specimens using 10% neutral buffered formalin and 96% alcohol in cell-block preparations, while other studies have described the practical application of alcohol-formalin fixation for body fluid cytology. These findings are consistent with the present observation that 10% formal alcohol maintained good nuclear–cytoplasmic contrast and staining uniformity (10-12).

From a practical laboratory perspective, the findings indicate that both fixatives can produce satisfactory Papanicolaou-stained pleural effusion smears. However, when background cleanliness is considered an important quality characteristic, 96% ethanol demonstrated a more favorable result in this study. A relatively clean background facilitates visualization of cellular structures and reduces potential interference during microscopic assessment.

Nevertheless, the findings should be interpreted within the scope of the study design. The present study evaluated staining quality using descriptive morphological criteria and did not assess diagnostic sensitivity, specificity, or diagnostic accuracy. Therefore, the results should not be interpreted as evidence that one fixative provides superior diagnostic accuracy over the other.

This study has several limitations. First, the number of specimens was relatively limited, which may restrict the generalizability of the findings. Second, staining quality was evaluated using categorical qualitative criteria without a validated semi-quantitative scoring system. Third, interobserver agreement was not assessed. Fourth, the study evaluated only conventional Papanicolaou staining and did not examine other preparations such as liquid-based cytology, cell-block preparations, or immunocytochemical procedures.

Future studies should consider larger sample sizes, standardized or validated scoring systems, assessment by more than one observer, and inferential statistical analysis when appropriate. Further research may also evaluate the performance of these fixatives in cell-block preparation, liquid-based cytology, and ancillary diagnostic techniques.

CONCLUSION

Both 96% ethanol and 10% formal alcohol produced good overall staining quality in Papanicolaou-stained pleural effusion smears under the conditions of this study. Both fixatives demonstrated comparable nuclear–cytoplasmic contrast and staining uniformity. However, 96% ethanol produced a higher proportion of smears with good background cleanliness than 10% formal alcohol (70% vs. 50%). Therefore, 96% ethanol remains the preferred fixative when optimal background cleanliness is required, while 10% formal alcohol may be considered a practical alternative for routine cytological preparation.

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