



Laboratory Journal of Infectious Diseases

Homepage: <https://jurnal.poltekkeskupang.ac.id/index.php/LJID>



Original Article

Cytological Characteristics of Pleural Effusion with Variations in 96% Alcohol Fixation Time Using Papanicolaou Staining

Priska Alicia Vansugisti ¹, Ary Nurmalasari ², Euis Tia Istianah ³, Rahayu Nirmatul Mutmainah ⁴

¹Medical Laboratory Technologist, STIKes Muhammadiyah Ciamis, Ciamis City, Indonesia

²Medical Laboratory Technologist, STIKes Muhammadiyah Ciamis, Ciamis City, Indonesia

³Medical Laboratory Technologist, STIKes Muhammadiyah Ciamis, Ciamis City, Indonesia

⁴Medical Laboratory Technologist, STIKes Muhammadiyah Ciamis, Ciamis City, Indonesia

ARTICLE INFO

Article history:

Received 18 April 2026

Revised 06 June 2026

Accepted 22 June 2026

Keywords:

96% Alcohol

Fixation time

Papanicolaou staining

Pleural effusion

Cytology

Corresponding author:

Ary Nurmalasari

Ciamis City

arynurmalasari@stikesmucis.ac.id

Doi:

ABSTRACT

Pleural effusion is a clinical condition that may indicate serious underlying diseases, including infection, malignancy, and systemic disorders. Cytological examination of pleural fluid plays an important role in determining the possible etiology of pleural effusion and requires proper fixation to preserve cellular morphology and staining quality. This study aimed to evaluate the cytological characteristics and preparation quality of pleural fluid specimens fixed in 96% alcohol for 15 minutes and 30 minutes using the Papanicolaou staining method. This quasi-experimental study included 16 pleural fluid samples obtained from routine laboratory examinations. Each sample was processed into four cytological smears, consisting of two duplicate smears fixed in 96% alcohol for 15 minutes and two duplicate smears fixed in 96% alcohol for 30 minutes. A total of 64 smears were evaluated, with 32 smears in each fixation group. All smears were stained using the Papanicolaou method and assessed microscopically based on background appearance, cellular morphology, nuclear characteristics, and overall staining quality. Of the 32 smears fixed for 15 minutes, 20 smears (62,5%) were classified as poor quality and 12 smears (37,5%) as good quality. In contrast, among the 32 smears fixed for 30 minutes, 3 smears (9,4%) were classified as poor quality and 29 smears (90,6%) as good quality. These findings indicate that fixation using 96% alcohol for 30 minutes produces better cytological preparation quality than fixation for 15 minutes. Therefore, a 30-minute fixation period is recommended to support optimal cytological evaluation and diagnostic accuracy.

Introduction

Pleural effusion is a common clinical condition and may indicate serious underlying diseases, including infections, malignancies, and systemic disorders. Globally, pleural effusion affects approximately 1,5 million individuals annually in the United States and has an estimated incidence of 320 cases per 100.000 population in industrialized countries (Jany & Welte, 2019; Ferreira et al., 2020; Tian et al., 2021).

In Indonesia, tuberculosis remains one of the leading causes of pleural effusion. According to the Ministry of Health of the Republic of Indonesia, tuberculosis cases increased from approximately 724.000 in 2022 to more than 809.000 in 2023, highlighting the importance of accurate diagnostic approaches for pleural fluid evaluation (Kemenkes, 2024; World Health Organization, 2023).

Citation:



© 2025 The Authors. Published xxx. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

Various laboratory examinations can be performed to determine the etiology of pleural effusion, including biochemical analysis, microbiological examination, and cytological evaluation. Among these methods, cytological examination plays an important role in identifying inflammatory processes and distinguishing benign from malignant cellular changes based on cellular morphology and nuclear characteristics (Chen et al., 2025).

The examination of pleural fluid to determine the cause of an effusion can be carried out through various analyses, including biochemical parameters such as pH, protein, glucose, triglycerides, albumin, and lactate dehydrogenase (LDH), as well as cell count and type analysis, Gram staining, microbiological culture, and cytological evaluation (Jany & Welte, 2019; Ferreiro et al., 2020). Among these methods, cytological examination plays a crucial role in identifying inflammatory processes and the aetiology of pleural effusions, including distinguishing between normal and malignant cells based on shape, size, intercellular relationships, and nuclear characteristics (Chen et al., 2025). The accuracy of cytological examination results is greatly influenced by an optimal fixation process, as fixation serves to preserve cell structure as close to its original state as possible, prevent damage or loss of cellular components, inhibit autolysis by intracellular enzymes, and enhance staining quality so that cellular morphological details can be observed more clearly (Suvana, Layton, & Bancroft, 2019).

Fixation is a crucial stage in cytological examination that is influenced by various factors, particularly duration; therefore, this process must be carried out immediately after the specimen is collected to prevent cell damage. The optimal fixation time depends on the type of fixative solution used, as each has a different ability to preserve cell structure. If carried out for too long, fixation can cause morphological changes such as cell shrinkage and wrinkling, which affect the quality of the preparation. One of the fixatives commonly used in cytology is alcohol, which works through a process of dehydration by replacing the water inside the cells; however, inappropriate use can also cause shrinkage and reduce the clarity of the cytoplasm and cell nucleus (Suvana, Layton, & Bancroft, 2019).

Fixation using 96% alcohol for approximately 30 minutes is the standard procedure in cytological preparation to preserve the morphological integrity of cells. However, several studies indicate that a shorter fixation time, such as 15 minutes, can still be used as an alternative under certain conditions to preserve cell structure, although its effectiveness in producing optimal specimen quality tends to be lower compared to the standard duration (Anggraini & Mahtuti, 2025; Dani & Sari, 2022; Ferreiro et al., 2020).

Although fixation with 96% alcohol for approximately 30 minutes is widely recommended as the standard procedure in cytological preparation, shorter fixation periods may be required in routine laboratory practice due to workload, specimen volume, or time constraints (Dey, 2018). However, information regarding the effect of reduced fixation duration on the quality of pleural fluid cytology preparations remains limited. Determining whether a shorter fixation period can maintain acceptable cytological quality is important to support laboratory efficiency while ensuring diagnostic reliability.

Therefore, this study aimed to evaluate the cytological characteristics and preparation quality of pleural fluid specimens fixed in 96% alcohol for 15 minutes and 30 minutes using the Papanicolaou staining method.

Methods

Study Design

This study employed a quasi-experimental post-test only comparative design to evaluate the effect of fixation duration on the quality of pleural fluid cytology preparations stained using the Papanicolaou method.

Study Setting and Samples

The study was conducted at Ciamis Regional General Hospital between January and May 2024. The study included 16 pleural fluid samples obtained from routine laboratory examinations. Consecutive sampling was applied during the study period.

Samples with sufficient volume and adequate cellular material for cytological preparation were included in the study. Samples with inadequate volume, severe cellular degeneration, or extensive contamination were excluded.

Preparation, Fixation, and Staining Procedures

Each pleural fluid sample was processed into four cytological smears. Two duplicate smears were fixed in 96% alcohol for 15 minutes (Group A), while the remaining two duplicate smears were fixed in 96% alcohol for 30 minutes (Group B). Therefore, a total of 64 cytological smears were evaluated, consisting of 32 smears in each fixation group.

All smears were stained using the Papanicolaou staining method and examined microscopically by a specialist in anatomical pathology. Alcohol fixation is commonly used in cytological preparation because it preserves cellular morphology, prevents autolysis, and supports optimal nuclear and cytoplasmic staining quality (Suvana, Layton, & Bancroft, 2019).

Cytological Quality Assessment

Cytological preparation quality was assessed based on four parameters: background appearance, cellular morphology, nuclear characteristics, and overall staining quality. These parameters are commonly used in cytological evaluation to determine the adequacy and interpretability of cytology preparations (Cibas & Ducatman, 2020; Koss & Melamed, 2006).

Each parameter was scored as 1 (poor) or 2 (good) according to the scoring system proposed by Thakur & Guttikonda (2017). Total scores ranged from 4 to 8. Preparations with scores of 4 were categorized as poor quality, whereas preparations with scores of 5–8 were categorized as good quality.

Results

A total of 64 cytological smears derived from 16 pleural fluid samples were evaluated in this study. Thirty-two smears were fixed in 96% alcohol for 15 minutes (Group A), and thirty-two smears were fixed in 96% alcohol for 30 minutes (Group B).

In Group A, 20 smears (62,5%) were classified as poor quality, while 12 smears (37,5%) were classified as good quality. Poor-quality smears in this group were characterized by a haemorrhagic or unclear background, visible artefacts, indistinct cellular morphology, unclear nuclear characteristics, and uneven staining intensity.

In Group B, 3 smears (9,4%) were classified as poor quality, whereas 29 smears (90,6%) were classified as good quality. Most smears in this group showed clearer backgrounds, better-preserved cellular morphology, more distinct nuclear characteristics, and more uniform staining results.

These findings indicate that cytological smears fixed in 96% alcohol for 30 minutes showed better preparation quality than those fixed for 15 minutes.

Table 1. Quality Assessment of Pleural Fluid Cytology Smears Fixed in 96% Alcohol for 15 Minutes

Criteria	N	Total Number	%	Description
Suboptimal:				
Skor 4	20	20	62,5	Bad
Optimal:				
Skor 5	7	12	37,5	Good
Skor 6	2			
Skor 7	3			

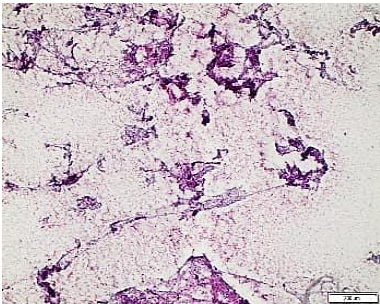
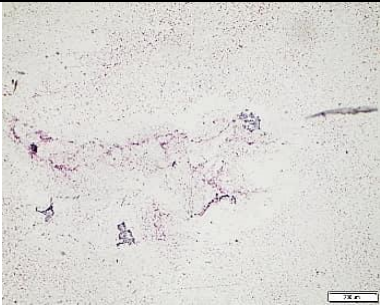
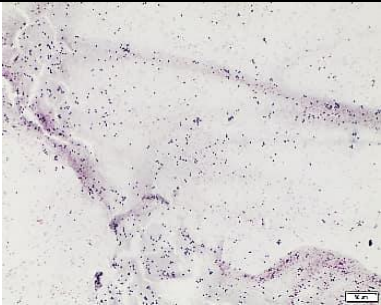
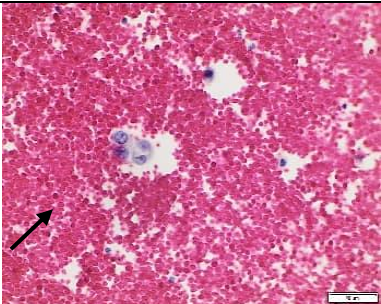
Table 1 shows the results of the quality assessment of pleural fluid cytology smear in Group A, which were fixed in 96% alcohol for 15 minutes; 20 smear (62,5%) received a 'poor' score and 12 smear (37,5%) received a 'good' score.

Table 2. Quality Assessment of Pleural Fluid Cytology Smears Fixed in 96% Alcohol for 30 Minutes

Criteria	N	Total Number	%	Description
Suboptimal:				
Skor 4	3	3	9,4	Bad
Optimal:				
Skor 5	4	29	90,6	Good
Skor 7	9			
Skor 8	16			

Table 2 shows the results of the quality assessment of pleural fluid cytology smear in Group B, which were fixed in 96% alcohol for 30 minutes: 3 smear (9,4%) received a 'poor' score and 29 smear (90,6%) received a 'good' score. The quality of pleural fluid cytology smear fixed in 96% alcohol for 30 minutes was found to be good.

Table 3. Representative Microscopic Findings of Pleural Fluid Cytology Smears After 15-Minute and 30-Minute Fixation in 96% Alcohol

Assessment criteria	Sample Code	
	Fixed in 96% Alcohol for 15 Minutes (A)	Fixed in 96% Alcohol for 30 Minutes (B)
Background Haemorrhagic (haemorrhagic, non-clear, and artefacts visible). Clear (Non-haemorrhagic, clear/transparent, and no artefacts visible).	 Slide code A14 (4x magnification) Not clear	 Slide code B12 (4x magnification) Clear and free of artefacts
Cell Morphology Poor (Cell shape and cytoplasmic colour intensity are unclear). Good (Cell shape and cytoplasmic colour intensity are clear).	 Slide code A8 (4x magnification) Not clear and artefacts are visible	 Slide code B16 (10x magnification) Clear and free of artefacts
	 Slide code A14 (40x magnification) Cell morphology and cytoplasmic colour intensity are indistinct	 Slide code B12 (40x magnification) Mesothelial cells Cell shape and cytoplasmic staining intensity are distinct

Discussion

The present study demonstrated that fixation duration influences the quality of pleural fluid cytology preparations stained using the Papanicolaou method. Preparations fixed in 96% alcohol for 30 minutes showed superior quality compared with those fixed for 15 minutes, as reflected by clearer backgrounds, improved cellular morphology, better nuclear preservation, and more uniform staining quality.

Fixation is a critical step in cytological examination because it preserves cellular morphology and prevents autolysis. Adequate alcohol fixation facilitates protein coagulation and cellular dehydration, thereby maintaining nuclear and cytoplasmic integrity during the staining process. Inadequate fixation may result in incomplete protein coagulation, cellular shrinkage, blurred nuclear detail, and uneven stain uptake, ultimately reducing specimen quality and interpretability (Suvarna, Layton, & Bancroft, 2019).

In the present study, preparations fixed for 15 minutes frequently exhibited haemorrhagic backgrounds, artefacts, and less distinct cellular features. These findings suggest that shorter fixation duration may not provide sufficient time for optimal preservation of cellular structures. Consequently, staining quality and morphological evaluation may be compromised.

In contrast, preparations fixed for 30 minutes demonstrated clearer backgrounds and more distinct nuclear and cytoplasmic characteristics. These findings are consistent with the standard cytological fixation protocol recommended for Papanicolaou staining and support previous findings reported by Dani & Sari (2022) and Anggraini & Mahtuti (2025), who observed improved staining quality and reduced artefact formation following adequate alcohol fixation.

From a practical laboratory perspective, these findings support the continued implementation of the standard 30-minute fixation protocol for routine pleural fluid cytology examinations. Consistent fixation duration may contribute to improved diagnostic reliability, enhanced quality assurance, and reduced interpretation errors during microscopic evaluation.

This study has several limitations. First, the number of pleural fluid samples was relatively small and originated from a single institution, which may limit the generalizability of the findings. Second, cytological quality assessment was based on observer interpretation despite the use of standardized scoring criteria. Third, only one alcohol concentration and two fixation durations were evaluated. Future studies involving larger sample sizes and additional fixation conditions are recommended to further validate these findings.

Conclusions

Fixation duration affects the quality of pleural fluid cytology preparations stained using the Papanicolaou method. Preparations fixed in 96% alcohol for 30 minutes demonstrated better cytological quality than those fixed for 15 minutes, characterized by clearer backgrounds, improved cellular morphology, better nuclear preservation, and more uniform staining results. Therefore, fixation in 96% alcohol for 30 minutes is recommended to support optimal cytological evaluation and diagnostic accuracy.

Author contributions

All authors participated in manuscript preparation. All authors have read and agreed to the published version of the manuscript.

Acknowledgements

The authors would like to express their gratitude to the ketua STIKes Muhammadiyah Ciamis, the Head of the DIII Medical Laboratory Technology Study Program at STIKes Muhammadiyah Ciamis; Head of RSHS Bandung.

Funding

There is no specific funding for this study.

Ethical approval statement

This study used residual pleural fluid specimens obtained from routine laboratory examinations and did not involve additional specimen collection or direct patient intervention. All patient identities were anonymized prior to analysis to ensure confidentiality. The study was conducted in accordance with institutional regulations and the principles of the Declaration of Helsinki.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary materials

No supplementary material available.

References

- Anggraini, F., & Mahtuti, E. Y. (2025). Comparison Cytological Staining Results of Pleural Effusion with Delayed Time without Alcohol Fixative and with Alcohol Fixative. *Jurnal Biosains*, 11(3). <https://doi.org/10.24114/jbio.v11i3.68026>
- Chen, X., Li, Y., Wang, H., & Wen, K. (2025). Evaluation of Cytomorphological Examination in the Diagnosis of Pleural Effusion. *Clinical and Experimental Medicine*, 25(1), 112. <https://doi.org/10.1007/s10238-025-01642-x>
- Cibas, E. S., & Ducatman, B. S. (2020). *Cytology: Diagnostic Principles and Clinical Correlates Fifth Edition*. Elsevier. Retrieved from: <https://shop.elsevier.com/books/cytology/cibas/978-0-323-63636-0>
- Dani, A. S. R., & Sari, I. (2022). Perbedaan Hasil Fiksasi Alkohol 96% Selama 15 Menit dan 30 Menit pada Pewarnaan Papanicolaou. *Journal of Indonesian Medical Laboratory and Science (JoIMedLabS)*, 3(2), 119-132. <https://doi.org/10.53699/joimedlabs.v3i2.84>
- Dey, P. (2018). *Basic and Advanced Laboratory Techniques in Histopathology and Cytology*. Springer. Retrieved from: <https://link.springer.com/book/10.1007/978-981-19-6616-3>
- Ferreiro, L., Toubes, M. E., San José, M. E., Suárez-Antelo, J., Golpe, A., & Valdés, L. (2020). Advances in Pleural Effusion Diagnostics. *Expert review of respiratory medicine*, 14(1), 51-66. <https://doi.org/10.1080/17476348.2020.1684266>
- Jany, B., & Welte, T. (2019). Pleural Effusion in Adults—Etiology, Diagnosis, and Treatment. *Deutsches Ärzteblatt International*, 116(21), 377. [10.3238/arztebl.2019.0377](https://doi.org/10.3238/arztebl.2019.0377)
- Kemkes. (2024). *Profil kesehatan Indonesia 2023*. Kemkes. Retrieved from: <https://kemkes.go.id/id/profil-kesehatan-indonesia-2023>
- Koss, L. G., & Melamed, M. R. (2006). *Koss' Diagnostic Cytology and its Histopathologic Bases Fifth Edition*. Lippincott Williams & Wilkins. Retrieved from: [https://books.google.co.id/books?hl=id&lr=&id=yUvhjShhpEC&oi=fnd&pg=PA1023&dq=Koss,+L.+G.,+%26+Melamed,+M.+R.+\(2020\).+Koss%E2%80%99+diagnostic+cytology+and+its+histopathologic+bases+\(6th+ed.\).+Elsevier.&ots=CQhNJnd6B&sig=2T1F1WX4QmC5X55NRDGLH5Apw5U&redir_esc=y#v=onepage&q&f=false](https://books.google.co.id/books?hl=id&lr=&id=yUvhjShhpEC&oi=fnd&pg=PA1023&dq=Koss,+L.+G.,+%26+Melamed,+M.+R.+(2020).+Koss%E2%80%99+diagnostic+cytology+and+its+histopathologic+bases+(6th+ed.).+Elsevier.&ots=CQhNJnd6B&sig=2T1F1WX4QmC5X55NRDGLH5Apw5U&redir_esc=y#v=onepage&q&f=false)
- Suvarna, K. S., Layton, C., & Bancroft, J. D. (2019). *Bancroft's Theory and Practice of Histological Techniques Eight Edition*. Elsevier. Retrieved from: [https://books.google.co.id/books?hl=id&lr=&id=CERPDwAAQBAJ&oi=fnd&pg=PP1&dq=Suvarna,+S.+K.,+Layton,+C.,+%26+Bancroft,+J.+D.+\(2023\).+Bancroft%E2%80%99s+theory+and+practice+of+histological+techniques+\(9th+ed.\).+Elsevier.&ots=yu-MzqgnwLU&sig=K_dorIfKdLF3mG9CWRruQp9f4uQ&redir_esc=y#v=onepage&q&f=false](https://books.google.co.id/books?hl=id&lr=&id=CERPDwAAQBAJ&oi=fnd&pg=PP1&dq=Suvarna,+S.+K.,+Layton,+C.,+%26+Bancroft,+J.+D.+(2023).+Bancroft%E2%80%99s+theory+and+practice+of+histological+techniques+(9th+ed.).+Elsevier.&ots=yu-MzqgnwLU&sig=K_dorIfKdLF3mG9CWRruQp9f4uQ&redir_esc=y#v=onepage&q&f=false)
- Thakur, M., & Guttikonda, V. R. (2017). Modified Ultrafast Papanicolaou Staining Technique: a Comparative Study. *Journal of cytology*, 34(3), 149-153. https://doi.org/10.4103/JOC.JOC_23_16
- Tian, P., Qiu, R., Wang, M., Xu, S., Cao, L., Yang, P., & Li, W. (2021). Prevalence, Causes, and Health Care Burden of Pleural Effusions Among Hospitalized Adults in China. *JAMA Network Open*, 4(8), e2120306. <https://doi.org/10.1001/jamanetworkopen.2021.20306>
- World Health Organization. (2023). *Global Tuberculosis Report 2023*. World Health Organization. Retrieved from: <https://www.who.int/publications/i/item/9789240083851>