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Comparison of Diff-Quick and Papanicolaou Staining Quality in Exudative Pleural Effusion Cytology at Buleleng Regional General Hospital

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ABSTRACT

Exudative pleural effusion cytology is an important diagnostic supporting examination used to evaluate cellular abnormalities in the pleural cavity. The quality of cytological staining plays a critical role in ensuring accurate interpretation of cellular morphology and pathological changes. This study aimed to compare the staining quality between Diff-Quick and Papanicolaou methods in exudative pleural effusion cytology at Buleleng District General Hospital. This study employed a descriptive analytic design with a comparative approach. The samples consisted of 32 exudative pleural effusion cytology slides stained using Diff-Quick and Papanicolaou methods. Staining quality assessment was based on four parameters, including cellular morphology, cytoplasmic staining intensity, nuclear staining intensity, and background clarity. Data were analyzed using the Wilcoxon signed-rank test. The results demonstrated a statistically significant difference between Diff-Quick and Papanicolaou staining methods ($p = 0.004$; $p \leq 0.05$). Papanicolaou staining showed superior visualization of cellular morphology, nuclear detail, and background clarity, whereas Diff-Quick staining provided relatively satisfactory cytoplasmic staining intensity. Overall, the Papanicolaou staining method produced better staining quality compared to the Diff-Quick method in exudative pleural effusion cytology. These findings indicate that Papanicolaou staining remains more effective for detailed cytomorphological evaluation in pleural effusion specimens.

Keywords: Diff-Quick, Exudative Pleural Effusion, Papanicolaou, Cytology, Staining Quality

ABSTRAK

Sitologi efusi pleura eksudatif merupakan salah satu pemeriksaan penunjang diagnostik yang penting dalam mengevaluasi kelainan seluler pada rongga pleura. Kualitas hasil pewarnaan sitologi berperan penting dalam menentukan ketepatan interpretasi morfologi sel dan perubahan patologis. Penelitian ini bertujuan untuk membandingkan kualitas hasil pewarnaan *Diff-Quick* dan *Papanicolaou* pada sitologi efusi pleura eksudatif di RSUD Kabupaten Buleleng. Penelitian ini menggunakan desain deskriptif analitik dengan pendekatan komparatif. Sampel penelitian terdiri dari 32 preparat sitologi efusi pleura eksudatif yang diwarnai menggunakan metode *Diff-Quick* dan *Papanicolaou*. Penilaian kualitas pewarnaan dilakukan berdasarkan empat parameter, yaitu morfologi sel, intensitas pewarnaan sitoplasma, intensitas pewarnaan inti sel, dan kejernihan latar belakang preparat. Analisis data dilakukan menggunakan uji *Wilcoxon signed-rank test*. Hasil penelitian menunjukkan adanya perbedaan yang signifikan antara hasil pewarnaan *Diff-Quick* dan *Papanicolaou* ($p = 0{,}004$; $p \leq 0{,}05$). Pewarnaan *Papanicolaou* memberikan visualisasi morfologi sel, detail inti, dan kejernihan latar belakang yang lebih baik, sedangkan pewarnaan *Diff-Quick* menunjukkan hasil yang cukup baik pada intensitas pewarnaan sitoplasma. Secara keseluruhan, metode pewarnaan Papanicolaou menghasilkan kualitas pewarnaan yang lebih baik dibandingkan metode *Diff-Quick* pada sitologi efusi pleura eksudatif. Temuan ini menunjukkan bahwa pewarnaan Papanicolaou masih lebih efektif untuk evaluasi sitomorfologi secara detail pada spesimen efusi pleura.

Kata kunci: *Diff-Quick*, Effusi *pleura* eksudatif *, Papanicolaou*, Pewarnaan.

INTRODUCTION

Pleural effusion is a condition characterized by excessive accumulation of fluid in the pleural cavity and commonly occurs as a secondary manifestation of various diseases, including infections, malignancies, heart failure, and inflammatory disorders(1,2). Based on fluid characteristics, pleural effusion is classified into transudative and exudative types. Exudative pleural effusion is frequently associated with serious pathological conditions such as tuberculosis and malignancy, thereby requiring accurate and reliable diagnostic examinations(3).

Cytological examination of pleural fluid is widely used as a diagnostic method because it is minimally invasive, relatively rapid, and has high diagnostic value in detecting malignant cells(4,5). One of the most critical stages in cytological examination is the staining process, as staining quality greatly influences the visualization of cellular morphology, nuclear structure, cytoplasmic characteristics, and background clarity of the specimen. Poor staining quality may lead to inaccurate interpretation and reduce diagnostic reliability.

Several staining methods are commonly used in pleural fluid cytology, including Diff-Quick and Papanicolaou staining. Diff-Quick staining is known for its rapid and practical procedure and provides good cytoplasmic contrast, making it useful for preliminary screening and rapid cytological evaluation(6). However, previous studies have reported that Diff-Quick staining has limitations in displaying nuclear details and background clarity, which are important components in cytomorphological interpretation. In contrast, Papanicolaou staining is considered the gold standard in cytological examination because it provides excellent nuclear detail, clearer chromatin patterns, and better overall cellular morphology, although the staining procedure is more complex and requires a longer processing time(7,8).

Differences in the characteristics of these staining methods may influence diagnostic interpretation and the accuracy of cytological evaluation. Previous studies have primarily focused on the diagnostic sensitivity of pleural fluid cytology or the effectiveness of staining methods in general cytopathology specimens. However, comparative studies specifically evaluating the staining quality of Diff-Quick and Papanicolaou methods in exudative pleural effusion cytology remain limited, particularly in regional hospital laboratory settings. Therefore, this study provides scientific novelty by specifically comparing the staining quality of Diff-Quick and Papanicolaou methods based on cellular morphology, cytoplasmic staining intensity, nuclear staining intensity, and background clarity in exudative pleural effusion specimens.

Understanding the comparative performance of these staining methods is important to support laboratory quality improvement and enhance diagnostic accuracy in cytological examinations. Therefore, this study aimed to compare the staining quality between Diff-Quick and Papanicolaou methods in exudative pleural effusion cytology at Buleleng District General Hospital. The findings of this study are expected to provide scientific evidence regarding the effectiveness of both staining methods and serve as a reference for selecting appropriate cytological staining techniques in clinical laboratory practice.

METHODS

This study employed a comparative analytical research design with a cross-sectional approach. The study was conducted at the Anatomical Pathology Laboratory of Buleleng District General Hospital from October to November 2025. The study population consisted of all exudative pleural effusion cytology slides examined at the Anatomical Pathology Laboratory of Buleleng District General Hospital during the period of May to August 2025. A total sampling technique was applied, resulting in 32 cytology slides that met the inclusion criteria. Each sample was stained using both Diff-Quick and Papanicolaou staining methods. The quality of staining was evaluated by one Anatomical Pathology specialist based on four assessment parameters: (1) cellular morphology, (2) cytoplasmic staining intensity, (3) nuclear staining intensity, and (4) background clarity of the slide preparation. Each parameter was assessed using an ordinal scale categorized as good or poor. Data analysis was performed using the Wilcoxon Signed-Rank Test because the data were not normally distributed. A p-value of ≤ 0.05 was considered statistically significant, indicating a meaningful difference between the staining quality of Diff-Quick and Papanicolaou methods in exudative pleural effusion cytology.

RESULTS

A total of 32 exudative pleural effusion cytology slides were evaluated to compare the staining quality between Diff-Quick and Papanicolaou methods. The assessment was performed based on four cytological parameters, including cellular morphology, cytoplasmic staining intensity, nuclear staining intensity, and background clarity.

Table 1. Quality Assessment of Diff-Quick Staining in Exudative Pleural Effusion Cytology

Parameter	Good n (%)	Poor n (%)	Total
Cellular morphology	23 (72%)	9 (28%)	32 (100%)
Cytoplasmic staining intensity	29 (91%)	3 (9%)	32 (100%)
Nuclear staining intensity	26 (81%)	6 (19%)	32 (100%)
Background clarity	24 (75%)	8 (25%)	32 (100%)

As presented in Table 1, Diff-Quick staining demonstrated the highest performance in cytoplasmic staining intensity, with 29 slides (91%) categorized as good quality. Nuclear

staining intensity was considered good in 26 slides (81%), while background clarity and cellular morphology showed good quality in 24 slides (75%) and 23 slides (72%), respectively. However, suboptimal cellular morphology and background quality were still observed in several preparations. These findings indicate that Diff-Quick staining provides satisfactory cytoplasmic contrast but may present limitations in preserving optimal cellular detail and background visualization.

Table 2. Quality Assessment of Papanicolaou Staining in Exudative Pleural Effusion Cytology

Parameter	Good n (%)	Poor n (%)	Total
Cellular morphology	31 (97%)	1 (3%)	32 (100%)
Cytoplasmic staining intensity	28 (88%)	4 (12%)	32 (100%)
Nuclear staining intensity	30 (94%)	2 (6%)	32 (100%)
Background clarity	31 (97%)	1 (3%)	32 (100%)

Table 2 demonstrates that Papanicolaou staining produced superior staining quality across most evaluation parameters. Good cellular morphology and background clarity were observed in 31 slides (97%), while nuclear staining intensity was categorized as good in 30 slides (94%). Cytoplasmic staining intensity also showed satisfactory results, with 28 slides (88%) categorized as good quality. Overall, Papanicolaou staining provided clearer cellular morphology, sharper nuclear detail, and cleaner background appearance compared to Diff-Quick staining.

Prior to comparative analysis, data normality was assessed using the Shapiro-Wilk test.

Table 3. Shapiro-Wilk Normality Test Results

Staining Method	p-value
Diff-Quick	0.000
Papanicolaou	0.000

The Shapiro-Wilk test demonstrated that both Diff-Quick and Papanicolaou staining data were not normally distributed ($p<0.05$). Therefore, non-parametric statistical analysis using the Wilcoxon Signed-Rank Test was performed to compare the staining quality between the two methods.

Table 4. Wilcoxon Signed-Rank Test for Overall Staining Quality

Staining Method	Mean Rank	p-value
Diff-Quick	7.187	0.004
Papanicolaou	7.750	

The Wilcoxon Signed-Rank Test revealed a statistically significant difference in overall staining quality between Diff-Quick and Papanicolaou methods ($p=0.004$; $p\leq0.05$). The higher mean rank observed in the Papanicolaou method indicates superior overall staining performance compared to Diff-Quick staining in exudative pleural effusion cytology.

Further comparative analysis was conducted for each staining parameter individually.

Table 5. Wilcoxon Signed-Rank Test Based on Staining Quality Parameters

Parameter	Diff-Quick Mean Rank	Papanicolaou Mean Rank	p-value
Cellular morphology	1.719	1.969	0.011
Cytoplasmic staining intensity	1.906	1.875	0.705
Nuclear staining intensity	1.812	1.937	0.102
Background clarity	1.750	1.969	0.008

As shown in Table 5, significant differences were identified in cellular morphology (p=0.011) and background clarity (p=0.008) between Diff-Quick and Papanicolaou staining methods. Papanicolaou staining demonstrated superior performance in preserving cellular morphology and providing cleaner background visualization. In contrast, no statistically significant differences were observed in cytoplasmic staining intensity (p=0.705) and nuclear staining intensity (p=0.102) between the two staining methods. Overall, these findings indicate that although Diff-Quick staining offers adequate cytoplasmic and nuclear staining characteristics, Papanicolaou staining provides significantly better overall cytological quality, particularly in terms of cellular morphology preservation and background clarity. These advantages may improve the accuracy of cytomorphological interpretation in exudative pleural effusion cytology specimens.

DISCUSSION

The present study compared the staining quality of Diff-Quick and Papanicolaou methods in exudative pleural effusion cytology based on four major cytological parameters, including cellular morphology, cytoplasmic staining intensity, nuclear staining intensity, and background clarity. The findings demonstrated that both staining methods were capable of producing diagnostically adequate cytological preparations; however, significant differences were observed in overall staining quality, particularly in cellular morphology preservation and background clarity.

Diff-Quick staining demonstrated relatively good performance, especially in cytoplasmic staining intensity, where 91% of the evaluated slides were categorized as having good cytoplasmic visualization. The strong cytoplasmic contrast observed in this study may be associated with the Romanowsky-based staining principle of Diff-Quick, which contains eosinophilic components capable of enhancing cytoplasmic detail and producing rapid color uptake. These findings are consistent with previous studies reporting that Diff-Quick staining provides superior cytoplasmic contrast and rapid visualization compared with several conventional cytological staining methods(9,10). The rapid processing time and practical staining procedure also make Diff-Quick advantageous for rapid on-site evaluation and preliminary cytological screening.

Despite its advantages in cytoplasmic staining, Diff-Quick demonstrated limitations in preserving optimal cellular morphology and background clarity. Only 72% of the slides exhibited good cellular morphology, while 25% showed suboptimal background quality. This limitation may be attributed to the air-drying fixation process used in Diff-Quick staining, which can induce cellular distortion and reduce structural preservation, particularly in exudative pleural effusion specimens containing abundant inflammatory cells, proteinaceous material, and cellular debris. In addition, the absence of sequential hydration and dehydration steps in Diff-Quick staining may contribute to the persistence of blood contamination and non-cellular debris within the preparation background. These findings support previous reports indicating that Diff-Quick staining often produces less clean background preparations compared to Papanicolaou staining (10). yang menyebutkan bahwa latar belakang pada pewarnaan *Diff-Quick* cenderung lebih kotor dibandingkan pewarnaan *Papanicolaou.*

In contrast, Papanicolaou staining demonstrated superior and more consistent staining quality across almost all evaluation parameters. Good cellular morphology was identified in 97% of the evaluated slides, indicating that Papanicolaou staining provides excellent preservation of cellular architecture. This superiority is likely associated with wet alcohol fixation and hematoxylin staining, which optimize nuclear preservation and maintain clear cellular boundaries. The findings of this study further support the established role of Papanicolaou staining as the gold standard method in cytopathological examination, particularly for detailed cytomorphological evaluation and malignancy assessment(8,10).

Papanicolaou staining also demonstrated excellent nuclear staining quality, with 94% of slides categorized as good quality. Clear chromatin patterns and well-defined nucleoli observed in this study are essential features in cytological interpretation, particularly in differentiating benign and malignant cellular changes. Similar findings have been reported by previous studies demonstrating that Papanicolaou staining provides superior nuclear detail compared to Diff-Quick staining in cytological specimens(11,12). Moreover, background clarity in Papanicolaou staining was significantly better, with 97% of slides demonstrating clean and interpretable backgrounds. The sequential hydration and dehydration procedures in Papanicolaou staining effectively reduce proteinaceous debris, erythrocytes, and inflammatory artifacts, thereby improving microscopic interpretation(11).

The comparative statistical analysis using the Wilcoxon Signed-Rank Test demonstrated a significant overall difference between Diff-Quick and Papanicolaou staining methods ($p = 0.004$). These findings indicate that both methods produce different staining characteristics in exudative pleural effusion cytology. Significant differences were identified in cellular morphology ($p = 0.011$) and background clarity ($p = 0.008$), where Papanicolaou staining showed significantly better performance than Diff-Quick staining. These results confirm that Papanicolaou staining is more effective in preserving cellular integrity and producing clearer preparation backgrounds, thereby supporting more accurate cytological interpretation(13).

Interestingly, no statistically significant differences were observed between the two staining methods in cytoplasmic staining intensity ($p = 0.705$) and nuclear staining intensity ($p = 0.102$). Although Diff-Quick descriptively showed stronger cytoplasmic staining contrast, while Papanicolaou demonstrated better nuclear visualization, both methods were considered diagnostically adequate for these parameters. These findings are consistent with previous reports indicating that Diff-Quick staining is advantageous for cytoplasmic evaluation, whereas Papanicolaou staining provides better nuclear and background detail(14). The novelty of this study lies in the specific evaluation of Diff-Quick and Papanicolaou staining quality exclusively in exudative pleural effusion cytology specimens. Previous comparative studies generally assessed mixed cytological specimens or focused primarily on diagnostic sensitivity without evaluating detailed staining quality parameters individually. In contrast, the present study comprehensively assessed four distinct cytological quality parameters and demonstrated that exudative pleural effusion specimens possess unique staining characteristics due to their complex inflammatory and protein-rich composition. This approach provides more specific evidence regarding the strengths and limitations of each staining method in pleural effusion cytology(15,16).

Furthermore, this study highlights the practical implications of staining selection in routine anatomical pathology laboratories, particularly in resource-limited settings. Diff-Quick staining remains valuable for rapid cytological assessment because of its simplicity, lower cost, and shorter processing time. However, for definitive cytomorphological evaluation, especially in suspected malignant pleural effusion, Papanicolaou staining remains the preferred method due to its superior preservation of cellular morphology and cleaner background visualization. High-quality staining is essential because accurate cytological interpretation directly influences diagnostic precision and clinical decision-making by anatomical pathologists.

Overall, the findings of this study reinforce the position of Papanicolaou staining as the gold standard technique for exudative pleural effusion cytology while also demonstrating that Diff-Quick staining may serve as a useful complementary method for rapid screening purposes. The results contribute important scientific evidence for improving cytological laboratory practices and optimizing staining selection according to diagnostic objectives and laboratory conditions.

CONCLUSION

This study demonstrated a statistically significant difference between Diff-Quick and Papanicolaou staining methods in exudative pleural effusion cytology. Overall, Papanicolaou staining produced superior staining quality compared to Diff-Quick staining, particularly in terms of cellular morphology preservation, nuclear staining clarity, and background cleanliness. These findings indicate that Papanicolaou staining provides more optimal cytomorphological visualization, which is essential for accurate cytological interpretation and diagnostic evaluation, especially in cases suspected of malignancy. Although Diff-Quick staining showed satisfactory performance in cytoplasmic staining intensity and offered advantages in terms of rapid processing and practicality, its limitations in maintaining cellular detail and background clarity reduce its effectiveness for comprehensive cytological evaluation. Therefore, Papanicolaou staining remains the gold standard staining method for body fluid cytology, particularly for exudative pleural effusion specimens requiring detailed morphological assessment. Future studies are recommended to involve larger sample sizes, additional cytological parameters, and comparisons with other staining techniques or ancillary diagnostic methods to further improve the quality and diagnostic accuracy of pleural effusion cytology examinations.

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