Bhakta Journal of Medicine. 2026. 1(1): 019-022

BHAKTA JOURNAL OF MEDICINE (BJM)

Available online at https://bjm.iik.ac.id/bjm

Diff-Quick Staining Effectiveness in 24-Hour Stored Diabetes Mellitus Urine

Indra Fauzi Sabban[1*)], Ratri Pramudita[2], Rizal Adiyta Hermawan[3], Jessi Suryani Setiawan[4], Ismiy Noer Wahyuni[5], Ronald Aprianto Parubak[6]

[1,2,3,4,5,6]Institut Ilmu Kesehatan Bhakti Wiyata Kediri

Article Info

Article History:

Received: 23/01/2026

Accepted: 04/03/2026

Published: 05/03/2026

*) Corresponding author:

E-mail: indra.fauzi@iik.ac.id

How to cite this article:

Sabban, I. F., *et. al*. (2026). *Diff-quick staining effectiveness in 24-hour stored diabetes mellitus urine*. Bhakta Journal of Medicine, 1(1), 19–22. https://bjm.iik.ac.id/bjm

Abstract

Diabetes Mellitus is a chronic metabolic disorder characterized by hyperglycemia due to abnormalities in insulin secretion, insulin action, or both. High blood glucose levels in patients with Diabetes Melitus can cause glucose to be detected in the urine (glucosuria), which reflects impaired renal filtration conditions. The urine of diabetic patients has certain characteristics that may affect laboratory examinations, including cytological examinations. Diff quick staining is one of the cytology staining methods that is derived from Romanowsky staining. This staining is widely used because the process is fast, efficient, and able to provide good color contrast in the nucleus and cytoplasm of cells. However, the quality of the staining results is greatly influenced by the sample conditions, including the time of the examination delay. This study aims to determine the impact of delaying the examination of urine samples for 24 hours on the quality of diff quick coloring in the urine of patients with Diabetes Mellitus. The study used a descriptive design with purposive sampling technique, involving 25 urine samples of type II Diabetes Melitus patients.The results showed that most of the urine samples had poor diff quick staining quality, even on the first day of sampling. A 24-hour storage delay caused a significant decrease in staining quality.This study concludes that delaying the examination of urine samples can affect the staining quality of urine samples.

Keywords: Diabetes Mellitus, Diff quick, Glucosuria, Cytology, Urine Sample.

Introduction

Diabetes Mellitus is a metabolic disorder characterized by hyperglycemia, a condition characterized by elevated blood sugar levels above normal levels. This can be caused by abnormalities in insulin secretion, insulin action, or both. This condition can cause long-term damage to organs, including the kidneys, known as diabetic nephropathy. Clinically, diabetic nephropathy is a complication that occurs in 40% of all diabetes mellitus patients and is the leading cause of kidney disease (Wulaniati & Prasetyawati, 2023). Urinary glucose is sugar present in urine due to impaired renal filtration. This is caused by a lack of the hormone insulin, which converts glucose into glycogen (Permanasari, 2024).

Urine sediment examination, or microscopic examination, is performed to identify organic and inorganic components in urine. Organic components include epithelial cells, leukocytes, erythrocytes, casts, and bacteria. Inorganic components include amorphous materials, crystals, and fatty substances (Parwati et al., 2022).

The Clinical and Laboratory Standards Institute (CLSI) recommends that urine tests be performed no later than two hours after collection. Delaying urine testing for two hours without storing it at 2-8°C and without adding preservatives can reduce the quality of the test results, especially the number of red blood cells in the urine.

One staining technique in cytology is the diff-quick staining method. Diff-quick staining is a derivative of Romanowsky staining and is used to stain cells from non-gynecological and blood samples, including Fine Needle Aspiration Biopsy (FNAB). The dye compositions used in diff-quick staining are eosin and methylene blue (Naid et al., 2014).

Based on this, researchers were interested in conducting urine cytology examinations in patients with Diabetes Mellitus, using urine that was directly examined and urine that had been stored for 24 hours. This study aimed to examine the results of diff-quick staining in both conditions. Despite existing guidelines emphasizing timely urine examination, there remains limited evidence regarding the specific impact of prolonged storage—especially 24-hour delays at room temperature—on the cytomorphological quality of urine samples stained with Diff-Quick in patients with Diabetes Mellitus. Most previous studies focus on routine urinalysis parameters or preservation methods, without specifically evaluating staining quality and cytological interpretation after delayed processing. This represents a critical research gap, particularly in clinical settings

with limited laboratory infrastructure where immediate examination is not always feasible.

Therefore, this study provides new evidence on the effect of a 24-hour examination delay on Diff-Quick staining quality in urine cytology from patients with Diabetes Mellitus, highlighting the consequences of delayed processing at room temperature without preservation. The novelty of this research lies in its comparative evaluation of directly examined urine specimens and specimens stored for 24 hours prior to Diff-Quick staining, with specific emphasis on cellular morphology, staining intensity, and interpretative reliability. The findings are expected to contribute to laboratory practice guidelines and improve the accuracy of cytological assessment in diabetic patients, particularly in resource-limited healthcare settings.

Materials and Methods

1. Research Design

This study used a descriptive observational design with a laboratory approach. This study aimed to describe in depth the morphological characteristics and quality of Diff-Quick staining results in urine sediment from patients with Diabetes Mellitus (DM) after a 24-hour delay in testing (sample stability) at room temperature.

2. Samples and Populations

Population: All individuals with diabetes mellitus who were registered and sought medical attention at Kediri City's Community Health Center made up the study's accessible population.

Sample: Twenty-five respondents who satisfied the inclusion requirements were chosen as the sample size.

Sampling Method: Purposive sampling combined with a non-probability sampling technique was employed. Patients having a diagnosis of diabetes mellitus, willingness to participate (informed consent), and the capacity to supply a sufficient, random urine sample (minimum 30-50 ml) were among the particular inclusion criteria used to select the sample.

3. Research Location and Time

Sampling: Conducted at Pesantren-1 Community Health Center in Kediri City.

Laboratory Analysis: Preparation, fixation, staining, and microscopic observation are performed in the Immunoserology Laboratory of the Bhakti Wiyata Kediri Institute of Health Sciences.

4. Laboratory Work Procedures (Complex)

To increase the validity of the results, the procedure is divided into several stages:

Pre-Analytics:

Collect a midstream urine sample in a sterile container.

Initial urine parameters are identified (urine glucose screening using a dipstick).

The sample is divided into two parts: one is examined immediately as a control (0 hour), and the other is stored for 24 hours at room temperature (20-25°C).

Analytics (Diff-Quick Staining Process):

Centrifugation: The urine is spun at 1,500-2,000 rpm for 5 minutes to obtain sediment.

Preparation: The supernatant is discarded, the sediment is collected, and a thin smear is made on a glass slide, then dried.

Staining:

Fix with Fixative solution (Methanol) for 1-2 minutes.

Dip in Eosinophilic solution (Solution I) 5-7 times.

Dip in Basophilic solution (Solution II/Methylene Blue) 5-7 times.

Rinsing: Rinse gently with running water or pH 6.8 buffer to remove any remaining dye.

Post-Analytical (Microscopic Observation):

Observations are made using a light microscope with 40x and 100x objective magnification (with oil immersion). Parameters observed include:

Cell Integrity: Whether epithelial cells, leukocytes, and erythrocytes are intact or lysed.

Dye Absorption Quality: The contrast between the cell nucleus and cytoplasm.

Identification of Organic Elements: The presence of bacteria or fungi (Candida), which often appear in DM urine after 24 hours.

5. Data Collection Instruments

Data were collected through laboratory observation sheets that recorded the staining quality categories (Good/Sufficient/Poor) and cell morphology (Intact/Damaged) as assessed by experts or through a scoring system based on scoring criteria.

6. Research Ethics

This research was conducted in accordance with the principles of health ethics, including providing informed consent to respondents, maintaining data confidentiality, and review by a health research ethics committee.

Results

This study was conducted to observe the staining results of urine stored for 24 hours at the Pesantren 1 Health Center in Kediri City, which is a patient with Diabetes Mellitus. The number of respondents in the study was 25 respondents who had met the requirements based on the inclusion criteria. In this study, the sample used was urine. Diff-quick staining of urine from Diabetes Mellitus stored for 24 hours was carried out in March 2025. The following are the results of the study, obtained from the diff-quick staining results of urine that was examined directly and stored for 24 hours.

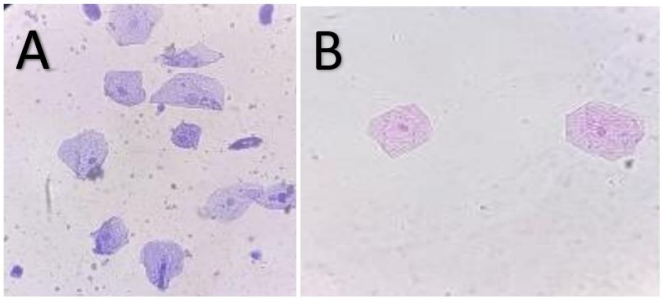

Figure 1. Microscopic examination, A. Results of 0-hour preparations and B. Delayed 24 hours using 40x magnification.

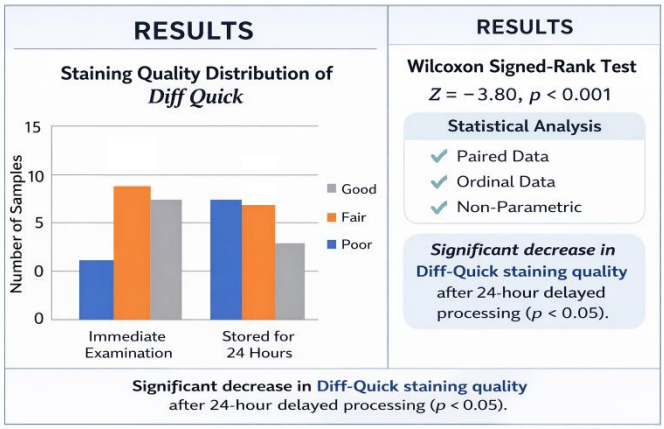

Figure 2. Graph and statistical results

Discussion

One of the staining techniques in cytology is the diff-quick method. Diff-quick staining is a derivative of Romanowsky staining and is used to stain cells from non-gynecological and blood samples, including Fine Needle Aspiration Biopsy (FNAB). The dye compositions used in diff-quick staining are eosin and methylene blue (Mutoharoh et al., 2020). Eosin is a synthetic dye belonging to the xanthene group. Eosin is acidic and binds to positively charged protein molecules in the cytoplasm and connective tissue. Eosin is a counterstain that can stain the cytoplasm and connective tissue. Methylene blue is an example of a cationic or positive ion stain in tissue. Cationic staining is also known as basic staining and basophilic staining. It is generally used to stain cell nuclei (Dila et al., 2023). The results of a study on the staining of urine samples using diff-quick staining showed that on the first day of sampling, 14 samples had fairly good results, while 10 samples had good results and 1 sample had poor results on the diff-quick staining. This indicates that most urine samples from patients with diabetes mellitus have fairly good diff-quick staining results. After the urine samples were stored for 24 hours, there was a significant decline in the quality of the staining results. The number of samples with poor results increased from 1 to 9, while the number of samples with good results decreased to only 3. This indicates that delaying urine sampling for 24 hours can significantly affect the quality of the diff-quick staining results (Naid et al., 2014; Parwati et al., 2022).

A good urine test should be performed when the urine is still fresh (less than 1 hour old), or no later than 2 hours after urination. A delay between urination and urine sample examination can affect specimen stability and the validity of the results (Riswanto & Rizki, 2015). The decline in the quality of Diff-Quick staining in urine samples from patients with diabetes mellitus stored for 24 hours is generally caused by degeneration of epithelial cell morphology due to autolysis, increased pH, and microbial growth. This is more significant when stored at room temperature without preservatives, where cell membrane degradation complicates cytological interpretation (Ahmed & Tom, 2011).

The results of Diff-Quick staining on urine smears show partially clearly visible cell morphology, with round cells, slightly basophilic cytoplasm, round nuclei with well-defined nuclear membranes, and smooth nuclear chromatin, although the background may still be dirty with artifacts. This is consistent with the literature (Selvi et al., 2001), which states that while Diff-Quick staining has these advantages, it has disadvantages such as less clear cell shape and cytoplasm, and a reddish-purple background. This study evaluated morphological staining quality but did not assess additional biochemical parameters, microbial growth, or long-term cytological stability beyond 24 hours. Future studies integrating cytomorphological, biochemical, and microbiological analyses would provide a more comprehensive evaluation of delayed urine processing effects. Despite these limitations, the study provides important preliminary evidence regarding the impact of 24-hour delayed examination on Diff-Quick staining quality in urine cytology.

Conclusion

Based on research on the results of diff quick urine staining, it can be concluded that delaying urine examination for 24 hours significantly reduces the quality of staining in patients with Diabetes Mellitus.

References

Ahmed, M., & Tom, M. (2011). The Consequence of Delayed Fixation on Subsequent Preservation of Urine Cells. Oman Medical Journal, 26(1), 12–15.

Clinical and Laboratory Standards Institute (CLSI). (2011). Urinalysis; Approved Guideline—Third Edition. CLSI document GP16-A3.

Dila, T. R., Raharjo, E. N., & Rukmana, D. I. (2023). Perbandingan pewarnaan giemsa, diff quick dan papanicolaou preparat efusi pleura di rsud a.w sjahranie. Jurnal Kesehatan Tambusai, 4(3), 4252–4258. https://journal.universitaspahlawan.ac.id/index.php/jkt/article/view/18768/14135

Mutoharoh, L., Santoso, S. D., & Mandasari, A. A. (2020). Pemanfaatan Ekstrak Bunga Sepatu (Hibiscus rosa-sinensis L.) Sebagai Alternatif Pewarna Alami Sediaan Sitologi Pengganti Eosin Pada Pengecatan Diff Quik. Jurnal SainHealth, 4(2), 21. https://doi.org/10.51804/jsh.v4i2.770.21-26

Naid, T., Mangerangi, F., & Almahdaly, H. (2014). Pengaruh Penundaan Waktu Terhadap Hasil Urinalisis Sedimen Urin. Jurnal Ilmiah As-Syifaa, 6(2), 212–219. https://doi.org/10.33096/jifa.v6i2.51

Parwati, P. A., Bintari, N. W. D., & Prihatiningsih, D. (2022). Penilaian Hasil Pemeriksaan Sedimen Urine Dengan Variasi Pengawet. Jurnal Inovasi Pendidikan, 3(3), 5445–5452.

Permanasari, N. G. (2024). Pengaruh penundaan waktu dan suhu pemeriksaan terhadap kadar glukosa urin dan protein urin 1. 140–150.

Riswanto, & Rizki, M. (2015). Urinalisis: Menerjemahkan pesan klinis urine. Yogyakarta: Pustaka Rasmedia.

Selvi, E., Manganelli, S., Catenaccio, M., De Stefano, R., Frati, E., Cucini, S., & Marcolongo, R. (2001). Diff Quik staining method for detection and identification of monosodium urate and calcium pyrophosphate crystals in synovial fluids. Annals of the Rheumatic Diseases, 60(3), 194–198. https://doi.org/10.1136/ard.60.3.194

Wulaniati, D. A., & Prasetyawati, D. (2023). Laporan Kasus: Seorang Perempuan dengan Asites Nefrogenik dan Diabetes Melitus Tipe 2. Roceeding Book Call for Papers Fakultas Kedokteran Universitas Muhammadiyah Surakarta, 415–421.

.