Optimization of Urine Storage with the Addition of 40% Formalin at a Temperature of 2–8°C: Stability of the Amount and Morphology of Sediment Elements

*Rusmini, Muhammad Ahyat Ridhoni

Medical Laboratory Technology Poltekkes Kemenkes Banjarmasin, Mistar Cokrokusumo Street 4A Banjarbaru Indonesia**.** *Email: rusmini.gbio14@gmail.com

Abstract: Urine sediment examination is an essential component of urinalysis used to detect various renal and urinary tract disorders. However, sediment elements in urine are prone to rapid degradation, requiring a preservation method that maintains both their quantity and morphology. This study aimed to evaluate the effect of urine storage with 40% formalin at 2–8°C for 3, 6, and 9 days on the mean count and morphology of erythrocytes, leukocytes, squamous epithelial cells, and transitional epithelial cells. A quasi-experimental design with a pretest–posttest control-group approach was used with six urine samples selected through purposive sampling. Data were analyzed descriptively, followed by repeated measures ANOVA and pairwise comparison tests. The results showed that erythrocytes (p=0.185) and transitional epithelial cells (p=0.775) did not exhibit significant differences across storage durations. Squamous epithelial cells showed significant differences in the ANOVA test (p=0.010), but the pairwise comparison did not yield consistent results. Leukocytes demonstrated significant differences in the ANOVA test (p=0.000), with a notable decrease observed on days 6 and 9 (p<0.05). Despite this, the morphology of all sediment elements remained well-preserved (score 2), with clearly distinguishable cell structures observable up to day 9. These findings suggest that urine preservation using 40% formalin at 2–8°C can maintain the count of erythrocytes, squamous epithelial cells, and transitional epithelial cells, while preserving the morphology of all four sediment elements for up to nine days. This has important implications for research requiring medium-term sample storage. The developed protocol can serve as a reference for standardizing urine preservation procedures.
Keywords: Cell morphology; formalin preservation; storage temperature (2–8°C); urine analysis; urine sediment.

INTRODUCTION

Urinalysis is a laboratory examination that includes macroscopic, chemical, and microscopic (urine sediment) analysis to provide screening information on kidney function, urinary tract conditions, and other metabolic disorders[1,2] Urine sediment examination requires samples containing organic elements such as erythrocytes, leukocytes, epithelial cells, and fungi, as well as inorganic components like crystals[3,4].

The first-morning urine, examined immediately after collection, is considered the most ideal sample for sediment analysis, as organic components typically remain stable for only 1–3 hours at room temperature[5]. Beyond this period, bacterial activity may

Corresponding Author: Rusmini
Medical Laboratory Technology Poltekkes Kemenkes Banjarmasin.
Mistar Cokrokusumo Street 4A Banjarbaru Indonesia.
Email: rusmini.gbio14@gmail.com

degrade urea, leading to cell lysis and hindering microscopic identification[6]. Previous studies have also reported a reduction in leukocyte count in urine stored at room temperature for just 1–6 hours[7]. These findings underscore the importance of pre-analytical factors, particularly sample storage conditions and duration, in ensuring the reliability of sediment analysis results.

Various preservation methods have been employed to slow down sediment degradation, including refrigeration at 2–4°C, which can maintain sample integrity for 24–48 hours, and the addition of preservatives such as formalin[5]. Previous research has shown that the addition of formalin to urine stored at room temperature for up to 9 hours does not significantly affect sediment components[8]. Furthermore, the use of preservatives such as formalin has also been reported to produce no significant difference in the number of urine sediment elements, including erythrocytes, leukocytes, epithelial cells, crystals, and bacteria[9]. Another study found that 40% formalin was able to preserve the type and quantity of urine sediment for up to 24 hours without significant differences compared to fresh samples[10].

However, most existing studies have focused on relatively short storage durations, providing limited insights into the effectiveness of combining refrigeration and formalin for longer-term preservation. Therefore, this study aims to evaluate the optimal duration of urine storage with the addition of formalin at 2–8°C for 3, 6, and 9 days, and to assess potential morphological changes in sediment elements. The findings are expected to offer an alternative long-term preservation method that maintains sediment morphology, thereby enabling more reliable microscopic examination.

MATERIALS AND METHODS

This study used a quasi-experimental, single-group pretest-posttest design. The Health Research Ethics Committee of the Bandung Polytechnic of Health, Ministry of Health, has approved this study with ethics number 168/KEPK-PKB/2025. Researchers used six urine specimens collected by purposive sampling based on specific inclusion criteria that matched the characteristics of the target population. Inclusion criteria included fresh urine samples from patients diagnosed with kidney disease who received four treatments: H1, H3, H6, and H9.

The study used conventional methods, with fresh urine as the dependent variable. Each urine sample was divided into four 10-mL containers. The first container (H1) was examined immediately and served as the control, without formalin added. The second (H3), third (H6), and fourth (H9) containers were each added with one drop of 40% formalin. Subsequently, the samples were stored at 2–8°C for 3, 6, and 9 days, respectively, which constituted the independent variable.

Urine sediment analysis was performed by transferring 8 ml of urine into a centrifuge tube. Samples were centrifuged for 5 minutes at 2000 rpm. The supernatant was discarded, and the remaining precipitate was homogenized. A 20 μL aliquot was pipetted onto a glass slide and covered with a cover glass. Sediment elements were observed under a light microscope using a 40× objective lens. Observations were recorded and averaged per high-power field (HPF).[9]

Morphological assessment of erythrocytes, leukocytes, squamous epithelial cells, and transitional epithelial cells was conducted using a 40× objective lens. The evaluation of sediment morphology was based on a scoring system assessing the clarity of shape

and cellular integrity. Scoring criteria (good, fair, and poor) were adapted from standard cell morphology characteristics in relevant literature (JCCLS)10 and verified by qualified healthcare professionals. The morphological scoring criteria are presented in Table 1.

Table 1. Scoring Table of Sediment Morphology Elements

No	Sediment Element	Morphological Description	Scale	Score
1	Erythrocyte	Biconcave disc, Ø ±7 µm, yellowish color clearly visible	Good	2
		Shape/size altered, color less clear	Fair	1
		Unclear or lysed	Poor	0
2	Leukocyte	Round, nucleated, granular, 1.5–2× the size of erythrocyte, clearly visible	Good	2
		Round, nucleated, granular, 1.5–2× the size of erythrocyte, less clear	Fair	1
		Unclear or lysed	Poor	0
3	Squamous Epithelium	Thin, flat, small round nucleus clearly visible, wide cytoplasm	Good	2
		Thin, flat, small round nucleus less clear, wide cytoplasm	Fair	1
		Unclear or lysed	Poor	0
4	Transitional Epithelium	Round/oval, large nucleus clearly visible	Good	2
		Round/oval, large nucleus less clear	Fair	1
		Unclear or lysed	Poor	0

Source: (JCCLS)[11]

Data analysis was conducted descriptively by calculating the mean number of sediment elements per HPF and assessing morphology using the scoring criteria. The average values per HPF were then analyzed using Repeated Measures ANOVA at a significance level of 0.05 to test data normality. This was followed by a Pairwise Comparison test to identify significant differences between storage durations and determine the optimal preservation condition.

RESULTS AND DISCUSSION

Table 2 presents the mean counts of erythrocytes, leukocytes, squamous epithelial cells, and transitional epithelial cells observed in six urine samples preserved with 40% formalin and stored at 2–8°C over various durations. The data include the average number per high-power field (HPF) and the morphology scores for each sediment element on days 1, 3, 6, and 9.

As shown in Table 2, the average erythrocyte count in the formalin-treated urine samples stored at 2–8°C was 5.38/HPF on day 1, decreasing to 4.35/HPF on day 3, 2.60/HPF on day 6, and 2.22/HPF on day 9. The leukocyte count also declined progressively from 25.65/HPF on day 1 to 14.15/HPF by day 9. Similarly, squamous epithelial cells decreased slightly from 3.27/HPF on day 1 to 2.47/HPF on day 9.

Transitional epithelial cells remained relatively stable, ranging from 1.0/HPF to 0.80/HPF throughout the storage period.

Table 2. Results of Average Examination of Erythrocytes, Leukocytes, Squamous Epithelium, and Transitional Epithelium

Sediment Element	Day	Mean (/HPF)	Morphology (0=poor, 1=pair, 2=good)
Erythrocytes	1	5.383	2
	3	4.35	2
	6	2.6	2
	9	2.217	2
Leukocytes	1	25.65	2
	3	23.167	2
	6	19.05	2
	9	14.15	2
Squamous Epithelium	1	3.267	2
	3	3.233	2
	6	2.767	2
	9	2.467	2
Transitional Epithelium	1	1.0	2
	3	0.817	2
	6	0.933	2
	9	0.8	2

Despite the reduction in cell counts, morphological preservation remained consistent across all elements. Erythrocytes maintained a morphology score of 2, characterized by clearly visible biconcave, non-nucleated disc shapes (Figure 1a). Squamous epithelial cells also retained a score of 2, with well-defined flat, irregular shapes, small round nuclei, and broad cytoplasm (Figure 1b).
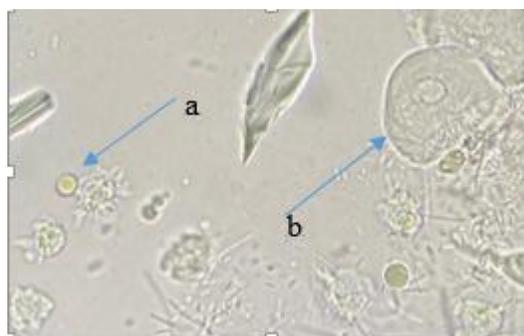

Figure 1. Morphology of Urine Sediment Cells.
(a) Erythrocytes with biconcave shape; (b) Squamous epithelial cells with clear cytoplasm and nucleus.

Leukocyte morphology remained intact up to day 9, with a consistent score of 2, showing clearly visible round, nucleated, and granular structures (Figure 2).

Figure 2. Morphology of Leukocytes.
Leukocytes preserved with visible nuclei and granules.

Similarly, transitional epithelial cells maintained a score of 2, exhibiting clear round or oval shapes, with prominent large nuclei and spindle-like structures (Figure 3).
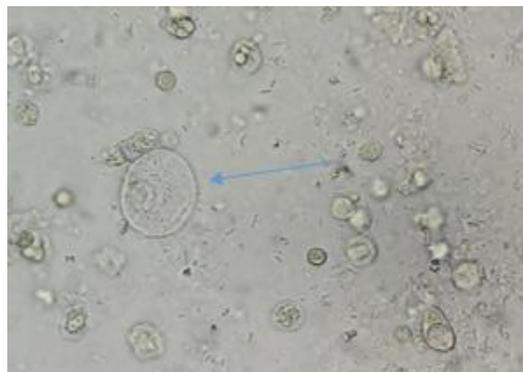

Figure 3. Morphology of Transitional Epithelial Cells.
Cells with oval nuclei and preserved cellular integrity.

All six urine samples (sp1, sp2, sp3, sp4, sp5, and sp6) preserved with 40% formalin and stored at 2–8°C for up to nine days demonstrated the continued presence of urine sediment elements, including erythrocytes, leukocytes, squamous epithelial cells, and transitional epithelial cells. To evaluate statistical significance across time points, repeated measures ANOVA was conducted. The results are summarized in Table 3.

The repeated-measures ANOVA showed that erythrocyte counts were normally distributed but lacked homogeneity (p = 0.185), indicating no significant differences across storage durations. Leukocyte data were non-normally distributed and thus log-transformed to meet homogeneity assumptions. The resulting p-value was 0.000,

indicating a statistically significant difference. Post hoc pairwise comparison revealed significant differences between day 1 and both day 6 and day 9 (p = 0.015 and 0.002, respectively), while the difference with day 3 was not significant.

Squamous epithelial data also required log transformation. ANOVA yielded a significant result (p = 0.010); however, pairwise comparisons showed no significant differences between individual days. For transitional epithelial cells, both log and square root transformations were applied to achieve homogeneity. The final p-value (p = 0.775) indicated no significant differences over time.

Table 3. Summary of Repeated Measures ANOVA Results

Sediment Element	Normality / Transformation	Homogeneity	ANOVA p-value	Pairwise Comparison (Day 1 vs Other Days)
Erythrocyte	Normal / –	Not Homogeneous	0.185	1 vs 3: 0.653; 1 vs 6: 1.000; 1 vs 9: 0.985
Leukocyte	Not normal → Log	Homogeneous	0.000 *	1 vs 3: 0.144; 1 vs 6: 0.015; 1 vs 9: 0.002
Squamous Epithelium	Not normal → Log	Homogeneous	0.010 *	1 vs 3: 1.000; 1 vs 6: 0.562; 1 vs 9: 0.296
Transitional Epithelium	Not normal → Log & Sqrt	Homogeneous	0.775	1 vs 3: 0.466; 1 vs 6: 1.000; 1 vs 9: 1.000

Note: * indicates statistically significant difference (p < 0.05).

Based on Table 3, the results of repeated measures ANOVA and pairwise comparisons showed no statistically significant differences in erythrocyte (p = 0.185) and transitional epithelial cell counts (p = 0.775) during storage from day 1 to day 9 at 2–8°C with the addition of 40% formalin. These findings are consistent with those of Sari DAI (2023), who reported that the use of formalin and toluene as preservatives did not significantly affect the mean number of erythrocytes or epithelial cells in urine, indicating the stability of sediment components during storage with formalin[9]. Similarly, Maharani et al. (2017) found that variations in formalin concentration from 10% to 37% did not affect either the quantity or type of urinary sediment[10].

In contrast, the repeated ANOVA results showed significant differences in leukocyte (p = 0.000) and squamous epithelial cell counts (p = 0.010). However, these differences were limited to specific day pairs and were not consistent. Pairwise comparisons of leukocytes revealed no significant change between day 1 and day 3, whereas significant decreases were observed on days 6 and 9 (p < 0.05). This indicates that storage beyond six days begins to affect leukocyte counts in urine. The decrease in leukocytes occurred more rapidly, likely due to their greater susceptibility to lysis compared to erythrocytes and epithelial cells, even with the addition of formalin. Leukocyte lysis is triggered by cell membrane damage caused by osmotic stress and

oxidative processes during storage. Residual activity of proteolytic enzymes and nucleases, although inhibited by formalin, can still contribute to leukocyte degradation[11]. Previous studies have also shown that refrigeration only slows but does not completely prevent chemical and cellular degradation in urine, leading to progressive deterioration over time[12]. Apart from that, several factors that disrupt the stability of sediment elements include specific gravity, the presence of microbes, and extreme urine pH, such as too acidic and too alkaline, which can also damage cell membranes 6. In contrast, pairwise comparisons for squamous epithelial cells revealed no significant differences across the observation days. These findings suggest that preservation with 40% formalin at 2–8°C remains effective in maintaining squamous epithelial cell stability.

Morphological observations of erythrocytes, leukocytes, squamous epithelial cells, and transitional epithelial cells were conducted using light microscopy and evaluated qualitatively based on a morphology scoring table. The findings indicated that no morphological alterations occurred in any of the four cell types following the addition of 40% formalin and storage at 2–8°C for up to nine days. These results align with other studies reporting that 40% formalin does not affect erythrocyte and leukocyte morphology[13]. Additionally, Johnson et al. (2021) demonstrated that the structure of vesicles and sediment cells in urine proteomics remained stable for up to eight days when stored at 4°C[14]. Preserving cellular morphology is critical, as structural distortion can hinder microscopic interpretation and affect clinical diagnoses. The combination of formalin and cold temperature offers dual protection: chemically via protein cross-linking and physically by slowing degradation at lower temperatures, thereby maintaining sediment integrity for extended periods.

Formalin is a formaldehyde solution often used as a sample preservative due to its ability to cross-link proteins by reacting with amino groups on amino acid residues such as lysine. This cross-linking stabilizes cellular and tissue structures by inhibiting enzymatic degradation and slowing microbial growth, thus maintaining the integrity of cellular and protein components in urine samples throughout the storage period[15]. Despite these preservation efforts, this study found that sediment quality began to decline after more than 3 days of storage, as indicated by a significant reduction in leukocyte count on days 6 and 9. This suggests that leukocytes are more susceptible to lysis during prolonged storage, even with the addition of formalin as a preservative.

This study has several limitations that should be considered. The relatively small sample size and the lack of repeat tests limited the test's statistical power. The pH of the urine samples was unknown, and the formalin concentration (40%) was too high, which affected the results of sediment element type and quantity observations. Nevertheless, this study can serve as a backup solution when immediate testing is not possible; the use of formalin and storage at low temperatures are valid and reliable conservation method.

CONCLUSION

This study concludes that urine storage with 40% formalin at 2–8°C for up to 9 days can maintain the morphology of erythrocytes, leukocytes, squamous epithelial cells, and transitional epithelial cells in good condition, while preserving the stability of erythrocyte and transitional epithelial cell counts. Leukocyte counts decreased significantly after more than 6 days of storage, while their morphological integrity remained intact. These findings indicate that the combination of refrigeration and formalin

effectively extends the storage period for urine, allowing sediment components to be clearly observed for microscopic analysis. This has important implications for research purposes that require medium-term sample storage. The developed protocol may serve as a reference for standardizing urine preservation procedures.

ACKNOWLEDGEMENT

The author would like to thank those who have helped carry out this research.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES
1. Gandasoebrata R. Penuntun laboratorium klinis. Jakarta: Dian Rakyat; 2013.
2. Niawaty P, Rikarni, Yulia D. Uji kesesuaian hasil pemeriksaan sedimen urine metode Shih-Yung pada volume urine 10 mL dan 5 mL. J Kesehat Andalas. 2021;10(2):88-93.
3. Naid T, Mangerangi F, Almahdaly H. Pengaruh penundaan waktu terhadap hasil urinalisis sedimen urin. As-Syifaa. 2014;6(2):212-219.
4. Subhan, Kristinawati E, Getas IW. Perbedaan jumlah kalsium oksalat urine metode sedimentasi antara kelompok vegetarian dengan non-vegetarian. J Analis Medika Bio Sains. 2019;6(2):105-111.
5. Susanti H, Yoavita, Rudianto, Puspitawati I. Pemeriksaan laboratorium urine rutin. Jakarta: Indonesian Association of Clinical Pathology and Medical Laboratory Specialists; 2022.
6. Delanghe J, Speeckaert M. Preanalytical requirements of urinalysis. Biochem Med (Zagreb). 2014;24(1):89-104.
7. Apriyani RK, Melani MS. Pemeriksaan jumlah leukosit pada pasien hiperglikemia menggunakan sampel urine dengan berbagai waktu penyimpanan. J Kesehat Tambusai. 2023;4(3):3238-3245.
8. Widiastuti UT. Pengaruh waktu tunda terhadap hasil sedimen urine menggunakan pengawet formaldehida [skripsi]. Semarang: Universitas Muhammadiyah Semarang; 2018.
9. Sari DAI. Differences in urine with sediment results preserving formalin and toluene. J Lab Khatulistiwa. 2023;6(2):212-219.
10. Maharani DMS, Inayat N, Wiwin M. Jenis dan jumlah sedimen urine menggunakan variasi konsentrasi pengawet formalin. J Kesehat. 2017;11(2).
11. Japanese Association of Medical Technologists, Editorial Committee of the Special Issue: Urinary Sediment. Aims of the guidelines on urinary sediment examination procedures proposed by the Japanese Committee for Clinical Laboratory Standards (JCCLS). J Jpn Assoc Med Technol. 2017;66:9-17.
12. Karakoyun I, Arslan FD, Baysoy A, Aksit M, Colak A, Isbılen Basok B. The effects of chlorhexidine-based preservative tubes on routine urinalysis stability. Int J Med Biochem. 2020;3(3):155-160.
13. Ribeiro KCB, Serafion BRL, Nolasco EL, et al. Urine storage under refrigeration preserves the sample in chemical, cellularity, and bacteriuria analysis of ACS. J Bras Patol Med Lab. 2013;49(6):415-422.

14. Johnson R, Patel A, Chen Y. Simple urine storage protocol for extracellular vesicle proteomics. Proteomics Clin Appl. 2021;15(5):e210002.
15. Salazar-García S, Lares-Villaseñor E, Bárcenas-Morales A, Vargas-Morales JM. Impact of chemical preservative in urine samples. EJIFCC. 2020;31(1):56-64.