JTL 15 (1) JUNE 2026
Page: 48-58

Contents list available at Jurnal Teknologi Laboratorium

JURNAL TEKNOLOGI LABORATORIUM

Journal Homepage: www.teknolabjournal.com
ISSN 2580-0191(Online) I ISSN 2338 – 5634(Print)

Original Research

Evaluation of tris–EDTA pre-hydrated non-FTA dried blood spot as a low-cost medium for room-temperature DNA sample transport

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Abstract: Dried Blood Spot (DBS) is a practical method for collecting DNA samples; however, DNA stability on non-FTA filter paper such as Whatman No. 42 remains limited. Pre-hydration of the DBS matrix with Tris–EDTA (TE) buffer has the potential to enhance DNA stability during room-temperature storage. This study aimed to evaluate the effectiveness of TE-hydrated DBS in maintaining DNA stability for short-term storage and transport. A paired quasi-experimental design was applied in which each subject provided two DBS samples: one on TE-hydrated paper and one on unmodified paper. Twenty subjects were recruited using convenience sampling, generating 40 DBS samples for analysis. TE hydration significantly increased DNA concentration compared with unmodified DBS ($p < 0.01$) and increased the proportion of samples with acceptable purity (A260/A280 = 1.8–2.0) ($p = 0.031$). Hydration with TE buffer was therefore effective in improving DNA yield, while its effect on DNA purity remained sensitive to technical variability and outlier influence. These findings indicate that TE-hydrated DBS has potential as an alternative medium for short-term DNA transport at room temperature. Further validation involving extended storage duration and downstream molecular testing is required.

Keywords: Dried blood spot; Tris–EDTA buffer; DNA stability; Room-temperature transport; Non-FTA filter paper.

INTRODUCTION

The DNA extraction process is a fundamental stage in molecular analysis that aims to obtain genetic material separated from other cellular components through a series of lysis, purification, and elution stages [1]. The technique for purifying compounds that have been extracted is called isolation [2]. In the context of molecular analysis, the next challenge after DNA extraction is ensuring the sample remains stable during the transport process. DNA sample transportation. There are several media that can be used such as EDTA tubes and DNA/RNA Shield. EDTA tubes require refrigeration during shipping and must be taken to the laboratory within 24-48 hours [3,4]. While DNA/RNA Shield allows room temperature transport and inactivate infectious agents such as viruses, bacteria, fungi, and parasites [5,6]. Although effective, DNA shields have the limitations of relatively high costs and limited availability.

Alternative DNA transport medium based on concept *dried needed* to provide more efficient and accessible options. This approach is widely known through the method Dried Blood Spot (DBS) was first introduced by Robert Guthrie

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DOI: 10.29238/teknolabjournal.v15i1.712
Received 03 December 2025; Received in revised form 05 December 2025; Accepted 04 January 2026

in 1963 as a dried biological sample [7]. As technology developed, DBS continued to innovate and is now widely used, including in molecular analysis [8,9].

DBS sampling has been widely explored for diagnostic and molecular analyses due to its logistical simplicity and stability without cold storage, provided that appropriate pre-analytical handling such as drying and packaging is applied [10,11]. Previous work also demonstrated that DNA extracted from DBS stored at room temperature remains sufficiently stable for PCR and other downstream applications. Studies of viral DNA stability on DBS further confirm minimal degradation over practical transport time frames [12].

[13] reported that DNA isolation from Whatman paper yielded highly variable DNA concentrations, ranging from 64.8–720 ng/µL. Although the DNA obtained was still suitable for further analysis such as PCR, this wide concentration range indicated instability of the results. This variability confirms that the DBS method still has limitations and requires further optimization.

Optimizing DNA yield from DBS requires modification in the form of hydration with Tris-EDTA buffer to increase DNA stability during room temperature storage. This buffer consists of Tris as a pH buffer and EDTA, which functions to chelate metal ions such as Mg^{2+}, which are important cofactors for nuclease enzymes [14,15]. By binding these ions, the activity of DNA-damaging enzymes can be inhibited, thus maintaining DNA stability and protection.

Most previous studies on DBS have focused on post-application optimization to improve DNA extraction efficiency. These efforts include modifying critical steps such as increasing lysis volume, adjusting centrifugation time and temperature, using cold isopropanol/ethanol, and selecting appropriate drying methods to achieve better purity, as well as adjusting the amount of starting material to obtain the most efficient protocol. Other studies have evaluated the effect of storage duration on DBS to assess DNA stability and determine the most effective isolation method [19,20]. However, matrix modification prior to blood application (pre-treatment) remains rarely explored, particularly for low-cost non-FTA filter papers such as Whatman No. 42, which lack chemical agents that protect DNA as found in commercial FTA cards.

Previous studies have reported the use of protease pre-treatment to enhance DNA release from DBS; however, such treatment is generally applied during the extraction stage rather than as an initial intervention intended to preserve DNA quality during storage [21,22]. In contrast, pre-hydrating the matrix using Tris-EDTA (TE) buffer as implemented in this study offers an alternative mechanism by providing a stable pH environment and a metal-ion chelating agent prior to blood application. This approach has the potential to mimic some protective functions of FTA cards in maintaining DNA integrity, yet with a more economical cost and feasible application to non-FTA paper. Thus, this research expands the limited body of work on non-FTA DBS pre-treatment and provides a preliminary foundation for the development of more affordable and practical room-temperature DNA transport media.

MATERIALS AND METHOD

Research Design

This study was conducted using a quasi-experimental design with a paired-comparison approach. Each participant contributed two dried blood spot (DBS) samples, one prepared on Whatman No. 42 filter paper pre-hydrated with Tris–EDTA (TE) buffer and the other on unmodified paper. The paired design enabled intra-subject comparison to minimize biological variability between individuals.

Study Population and Sampling

The study population consisted of eighth-semester students from the Medical Laboratory Technology Program at Poltekkes Kemenkes Palembang who

met the eligibility criteria. Participants were initially recruited using convenience sampling, followed by simple random selection among eligible volunteers to improve objectivity. A total of twenty participants were included, generating forty DBS samples. The experimental work was carried out at the Biotechnology Laboratory, Faculty of Medicine, Sriwijaya University, between March and April 2025.

Ethical Approval

Ethical approval was obtained from the Health Research Ethics Committee of Poltekkes Kemenkes Palembang (Approval No. 0062/KEPK/Adm2/II/2025). All procedures complied with the Declaration of Helsinki. Participants received detailed explanations regarding the study objectives, procedures, benefits, and potential risks, and written informed consent was obtained prior to blood collection. Participants were eligible if they were healthy eighth-semester students willing to participate and had not undergone blood transfusion within the previous three months. Individuals with a known history of hematologic disorders, coagulopathy, medication affecting blood quality such as anticoagulants or chemotherapy, or those unwilling to provide written consent were excluded from the study.

Sample Collection

Capillary blood was collected and applied to Whatman No. 42 filter paper discs. Prior to sample application, the filter paper designated for the experimental group was pre-hydrated with 50 µL of TE buffer based on preliminary saturation testing, which confirmed that this volume allowed uniform absorption across the 5 mm diameter spotting area without leaving excess moisture. The hydrated paper was dried under laminar airflow for one hour before use. Subsequently, 50 µL of capillary blood was pipetted onto each disc in both modified and unmodified conditions and allowed to dry for 24 hours. To ensure procedural consistency, all DBS samples were collected and processed by a single operator. After drying, the DBS samples were stored for three days at room temperature (22–27°C) in sealed ziplock bags without desiccant.

DNA extraction was performed from five 5 mm punches taken from each DBS sample using the Wizard® Genomic DNA Purification Kit (Promega) according to the manufacturer's instructions. DNA concentration and purity were measured using a Nanodrop One UV–Vis spectrophotometer with a 1 mm pathlength. Each sample was measured in duplicate to ensure consistency. Concentration values were obtained at 260 nm with background correction at 320 nm, while purity was expressed as the A260/A280 ratio automatically generated by the instrument without manual adjustment.

Data Analysis

Statistical analysis was carried out using SPSS with a significance level of 0.05. Data normality was assessed using the Shapiro–Wilk test. Since DNA concentration data were not normally distributed and derived from paired samples, differences between TE-hydrated and unmodified DBS were analyzed using the Wilcoxon signed-rank test. DNA purity values were categorized into acceptable and unacceptable based on the A260/A280 range of 1.8–2.0 and compared using McNemar's test for paired proportions. Concentration data are presented as median and interquartile range (IQR), while purity results are reported as frequencies and percentages.

Outlier detection was performed using the interquartile range method, where values falling below Q1 minus 1.5 times the IQR or above Q3 plus 1.5 times the IQR were classified as potential technical artefacts. These outliers were excluded in secondary analyses to improve the validity and reproducibility of purity interpretation.

RESULTS AND DISCUSSION

Table 1 showed the median DNA concentration in the DBS group with TE buffer hydration modification was recorded as higher at 3.05 ng/µL compared to the unmodified group at 1.90 ng/µL. The wider interquartile range (IQR) in the modified group at 3.40 indicates greater variation in DNA results between samples, while the unmodified group had a narrower IQR of 0.55, indicating a more homogeneous distribution of DNA concentrations but with a lower median value.

For DNA purity (A260/A280), the modified group showed a median close to the ideal range of 2.0 with an IQR of 0.51, while the unmodified group had a median of 3.24 with an IQR of 1.37, indicating data instability and the possibility of extreme values or measurement artifacts. These findings suggest that TE hydration modification not only increases the median DNA concentration but also results in more biologically consistent purity.

DNA purity values outside the normal biological range were identified as outliers using the IQR method and were removed from further analysis to maintain the validity of data interpretation. Possible causes for these extreme values are further explained in the discussion section.

Table 1. Descriptive statistics of DNA concentration and purity obtained from modified and unmodified dried blood spot (DBS) samples

Parameter	Group	N	Median	IQR	Min	Max
DNA concentration (ng/µL)	Modification	20	3.05	3.40	2.5	132.2
	No Modification	20	1.90	0.55	1.4	3.3
DNA Purity (A260/A280)	Modification	20	2.0	0.51	1.36	2.85
	No Modification	20	3.24	1.37	2.08	61.32

Table 2, showed that the DNA concentration in dried blood spot (DBS) samples that underwent a hydration process using Tris-EDTA (TE) buffer was significantly higher than that in unmodified DBS. Based on the Wilcoxon signed-rank test, the median DNA concentration in the TE hydration treatment was 3.05 ng/µL, while the DNA concentration without hydration was 1.90 ng/µL ($Z = -3.884$; $p < 0.001$). These findings indicate that hydration using TE buffer provides a significant increase in the amount of DNA extracted compared to the conventional method without hydration treatment.

Table 2. Results of the Effectiveness Test of DBS with TE Buffer Hydration Modification on DNA Concentration

Group	Median	IQR	Z	p-value
Modification	3.05	3.40	-3.884	0.001
No Modification	1.90	0.55		

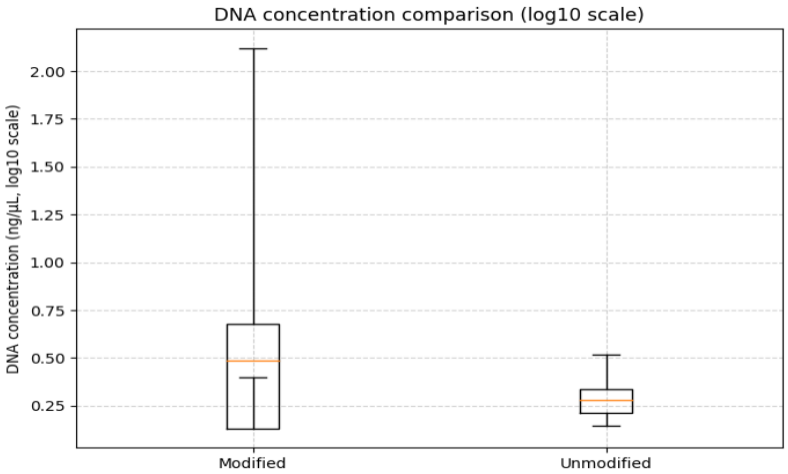

Figure 1. Comparison of DBS Concentration with Modification and Without Modification

The greater variation in DNA concentration in the DBS group with TE buffer hydration reflects the technical dynamics of the extraction process, which can indeed produce different yields between sample points, the researchers assume due to differences in hydration levels or lysis efficiency (figure 1). The higher concentration values are still within the biologically acceptable range and are consistent with the possibility of more optimal extraction in certain samples. This boxplot visualization confirms that TE hydration is able to increase DNA concentration, but is accompanied by greater variation between samples compared to the unmodified group.

Table 3. Distribution of DNA purity based on eligibility criteria

Group	Pure (1.80–2.00)	Impure (<1.80 or >2.00)	Total	p-value
Modification	6 (30%)	14 (70%)	20	0.031
No Modification	0 (0%)	20 (100%)	20	

Table 3, shows that there is a significant difference in the level of DNA purity between the two treatment groups.The A260/280 ratio indicates that DNA purity in modified DBS with a median value of 1.96, within the ideal range, although some samples were slightly higher than 2.0. In unmodified DBS, we found a median value of 3.24, which we identified as likely due to the suboptimal drying process of the pellet after ethanol aspiration, allowing for contamination.

The unmodified group exhibited extremely high purity values (maximum 61.32), which is biologically impossible for pure DNA. This value was identified as a technical artifact and excluded from the main analysis. Therefore, the analysis was performed by excluding outliers from the data. Outliers in DNA purity data were determined using the Interquartile Range (IQR) approach, with the outlier criteria being values below the lower limit (Q1 − 1.5×IQR) or exceeding the upper limit (Q3 + 1.5×IQR). In the DBS group with TE buffer hydration modification, the IQR calculation yielded a lower limit of 0.92 and an upper limit of 3.03, so values outside this range were categorized as outliers and excluded from further analysis. Meanwhile, in the group without modification, the lower limit was 0.93 and the upper limit was 5.49. The application of these thresholds ensures that statistical analyses are performed on a more representative data distribution, minimize technical distortion, and reflect more realistic biological conditions.

Table 4. Summary of Descriptive Statistics of DNA Concentration and Purity from Modified and Unmodified DBS After Outlier Exclusion

Parameter	Group	N	Median	IQR	Min	Max
DNA Purity (A260/A280)	Modification	17	2.00	0.50	1.36	2.85
	No Modification	17	3.17	1.04	2.08	4.93

Descriptive analysis of DNA purity based on the A260/A280 ratio revealed a significant change in distribution after outlier exclusion. Initially, the TE buffer hydration-modified DBS group had a median of 2.00 with an IQR of 0.51, while the unmodified group had a median of 3.24 with an IQR of 1.37. The very wide range of values in the unmodified group, including a maximum of 61.32, indicates the presence of biologically inconsistent extremes. After outlier removal, both groups displayed a more stable distribution that was representative of the real world. The median for the modified group became 2.00 with an IQR of 0.50, while the unmodified group showed a median that decreased to 3.17 with a narrower IQR of 1.04. These changes make it clear that the initial differences between the groups were largely influenced by extreme values, and that the post-exclusion data provide an alternative estimate of DNA purity for both biological and technical interpretation purposes.

Table 5. Distribution of DNA purity based on eligibility criteria after outlier exclusion

Group	Pure (1.80–2.00)	Impure (<1.80 or >2.00)	Total	p-value
Modification	5 (29.4%)	12 (70.6%)	17	0.063
No Modification	0 (0%)	17 (100%)	15	

Table 5 is a test results for DNA purity classification showed different interpretations when the analysis was performed on the entire data set compared to the data set without outliers. In the initial data (N=20), the Modified group showed a proportion of pure samples of 30%, while the Unmodified group produced no pure samples at all. This difference was statistically significant (p=0.031), indicating that the TE buffer hydration modification had an effect on increasing the proportion of pure DNA. However, after removing outliers (N=17), the proportion of purity in the Modified group became 29.4% and returned to 0% in the Unmodified group, but the significance value increased to p=0.063, thus no longer reaching the significance limit. This indicates that the presence of outliers also affects the sensitivity of the McNemar test.

DNA Concentration Quality

Based on the analysis results, DBS hydration using Tris-EDTA (TE) buffer significantly increased DNA concentration at room temperature, with a median of 3.05 ng/µL compared to the non-hydration group of 1.90 ng/µL ($Z = -3.884$, $p = 0.001$). This finding indicates that initial hydration with TE buffer is able to maintain the effectiveness of DBS in maintaining DNA concentration during storage and transportation.

These results are consistent with previous studies. [14] reported that TE plays a role in stabilizing pH and binding divalent metal ions, thereby preventing DNA degradation by nucleases. Montgomery (1990) in [24]stated that Tris functions as a buffer that maintains DNA stability by maintaining the pH at optimal conditions of around 7-8, while EDTA inactivates the DNase enzyme by binding Mg^{2+} and Ca^{2+} ions required as cofactors, thus maintaining DNA integrity. In addition, [25] showed that the use of TE buffer resulted in a higher DNA concentration of around 5% compared to PBS buffer, strengthening the evidence that TE is effective in increasing DNA yield from dry samples.

Furthermore, several studies on the use of dried blood spots in various diagnostic applications also support these findings. Certain paper membranes, such as Whatman, have proven effective in collecting biomaterial for laboratory testing, including in the diagnosis of HIV infection. In fact, comparative studies have shown that some types of membranes, such as the Munktell TFN, have high measurement efficiency.*viral load and* genotyping [26]. However, in a study conducted by [27], the results of genomic DNA isolation from DBS samples showed a relatively smaller amount of genomic DNA compared to venous blood samples. The electropherogram profile of genomic DNA from clinical DBS samples appeared to produce a rather faint and thin DNA band, confirming that the characteristics of the dry matrix and its preparation conditions, including the use of buffers such as TE, significantly influence the quantity and quality of the resulting DNA.

The findings of this study are consistent with previous research conducted by [28] which emphasized the important role of buffer solution type and storage temperature on DNA stability. Previous research reported that DNA stored at various temperatures remained intact for the first week of storage. However, DNA dissolved in distilled water and stored at room temperature (25 °C) began to degrade after two weeks and experienced complete degradation after four weeks. In contrast, DNA dissolved in Tris-EDTA (TE) buffer at the same temperature showed a slower degradation rate. At low temperature conditions (4 °C), DNA in distilled water began to degrade after four weeks, while DNA in TE buffer remained stable for up to ten weeks of storage. These results indicate that although DNA is generally considered a relatively stable molecule, environmental factors such as temperature and buffer type play a significant role in its integrity. Therefore, the use of TE buffer in this study supports DNA stability during storage and transport at room temperature, consistent with previous empirical findings.

However, the average DNA concentration across all samples remained low, a limitation of this study. This is likely due to the failure to optimize the extraction protocol, particularly regarding the reagent-to-sample ratio and the elution volume used. Several studies have shown that adjusting the elution volume plays a crucial role in increasing DNA concentration without increasing the starting material, making optimization of this step a crucial aspect of the DNA extraction procedure from dried blood spot (DBS) samples [29]

On the other hand, although the DBS group modified with TE buffer hydration showed a higher DNA concentration than the unmodified group, there was also greater variation between samples. This variability likely reflects differences in the uniformity of the hydration process or an interaction between the TE buffer and the chemical components of the extraction kit used. This finding confirms that successful DNA concentration enhancement depends not only on buffer selection but also on consistent technical control of the procedure. Thus, hydration using TE buffer proved effective in increasing DNA concentration from DBS, but further technical control and optimization are needed to obtain more stable and reproducible results.

As additional discussion material, it is necessary to consider the existence of environmental variables that cannot be fully controlled in this study and theoretically have the potential to affect the quality of the DNA produced. Temperature is an environmental factor that plays a significant role in influencing DNA stability. Increasing temperature can accelerate chemical reactions that damage covalent bonds in DNA molecules, potentially causing fragmentation and reduced genomic integrity [30]. Furthermore, the stability of the DNA double helix structure is also influenced by the physicochemical conditions of the solution, where hydrogen bonds between nitrogen bases can be broken at high temperatures or in solutions with low ionic strength [31]. Although DNA denaturation is reversible through the renaturation process at a certain temperature drop [32], uncontrolled temperature fluctuations during sample storage and transportation still have the potential to gradually degrade DNA quality.

DNA Purity Stability

Bivariate analysis using McNemar's test showed that there was a significant difference in DNA purity levels between the DBS group modified with TE buffer hydration and the unmodified group, with a p-value of 0.031 ($<\alpha = 0.05$). Although previously only 30% of samples in the modified DBS group showed a DNA purity ratio (A260/280) in the standard range of 1.80 -- 2.00, this statistical result indicates that hydration with TE buffer significantly increased the proportion of samples reaching the “pure” purity category.

These results are consistent with those reported by [25], who found that DNA from DBS stored at room temperature had lower purity than DNA from frozen fluid samples due to the presence of impurities in the paper. These findings confirm that although the absolute purity of DNA from DBS may be lower, pre-hydration with TE buffer can help maintain DNA integrity and increase the proportion of “pure” samples for further analysis. Furthermore, [20] showed that some isolation methods, including Wizard® Promega, tend to produce low purity values, indicated by electrophoregrams that show no bands. Therefore, these results emphasize that hydration with TE can maintain the purity of DNA from DBS, although the isolation procedure and sample characteristics still influence the final results.

In addition to determining DNA concentration, UV–Vis spectrophotometers also play a crucial role in detecting potential contaminants that could affect the A260/A280 ratio, such as proteins, phenols, or RNA remaining after the extraction process [33]. The presence of these contaminants has the potential to cause purity values to deviate outside the theoretical range of pure DNA, thus affecting the interpretation of statistical analysis results. However, this study has methodological limitations because the researchers did not have access

to complete absorbance spectrum data from the UV–Vis spectrophotometer. This lack of data limits the researchers' ability to definitively identify the source of the purity deviations, whether caused by specific contamination, optical interference, blanking errors, or other instrumental artifacts. Therefore, changes in statistical significance after outlier exclusion should be interpreted with caution, as they likely reflect the influence of technical factors in the measurement, rather than solely biological differences or the effectiveness of the TE buffer hydration treatment.

Research Limitations

The main limitation of this study is the absence of an RNAse step, which could lead to RNA contamination in the final results and affect the A260/A280 ratio. Furthermore, the kit protocol used was not specifically designed for DBS samples, so optimal results were not achieved. The absence of this optimization step also affected several processes in the extraction, including the pellet drying step in air (after ethanol aspiration), which could potentially cause contamination during this process. Nevertheless, the modified TE buffer hydration shows strong potential as a cost-effective approach for DNA transport at room temperature without the use of cooling media.

These findings open the door to the development of a practical, dried-matrix-based DNA transport method that can be implemented in areas with limited facilities. Further research is recommended to optimize the protocol, including reagent ratios and RNAse steps, and test its effectiveness over longer storage periods.

The results of this study indicate that DBS hydrated with TE buffer has the potential to be used as a short-term DNA transport medium at room temperature. However, the testing was only conducted for three days, making this duration too short to reflect transport conditions in many field situations. Furthermore, this study did not evaluate the performance of the DNA in further analyses such as PCR. Therefore, further research is needed to assess the effectiveness of longer-term storage and the suitability of the obtained DNA for subsequent molecular analysis applications.

In addition to determining DNA concentration, UV–Vis spectrophotometers also play a crucial role in detecting potential contaminants that could affect the A260/A280 ratio, such as proteins, phenols, or RNA remaining after the extraction process[33]. The presence of these contaminants has the potential to cause purity values to deviate outside the theoretical range of pure DNA, thus affecting the interpretation of statistical analysis results. However, this study also faced limitations related to access to raw absorbance data from the UV–Vis spectrophotometer. This limitation prevented researchers from conducting an in-depth investigation into the causes of variations in the A260/A280 ratio that fell far outside the theoretical range. Without separate absorbance data at the A260 and A280 wavelengths, a comprehensive evaluation of potential technical errors, such as unstable blanking, optical deviation of the cuvette, or instrument noise, could not be conducted.

CONCLUSION

Modification of the dried blood spot (DBS) method through pre-hydration of Whatman No. 42 filter paper with Tris–EDTA buffer demonstrated a significant increase in extracted DNA concentration compared with unmodified DBS. Although an improvement in the proportion of samples within the acceptable purity range was observed, this effect was influenced by technical variability and sensitivity to outlier data. These findings suggest that TE-hydrated DBS has potential as an economical alternative for short-term DNA storage and transport at room temperature. However, further validation involving longer storage duration and downstream molecular testing is required to confirm its broader applicability.

AUTHORS' CONTRIBUTIONS

PDZF contributed to conceptualization, methodology, investigation, data curation, formal analysis, and writing the original draft. AS contributed to methodology, validation, supervision, and writing review and editing. HD contributed to investigation, provision of resources, and data curation. O contributed to investigation, laboratory analysis, and data collection. ARP contributed to formal analysis, visualization, and writing review. SMA contributed to investigation, documentation, and data curation.

ACKNOWLEDGEMENT

The authors would like to express their sincere gratitude to the educational institutions, the institutions where the research was conducted, and the reagent grant providers for their support in the form of facilities, research permits, and reagent assistance that enabled this study to be carried out successfully.

FUNDING INFORMATION

The research reagents were supported by the PDUPT Grant of the Ministry of Health of the Republic of Indonesia for the 2023–2024 period and by the Medical Laboratory Technology (TLM) Laboratory of Universitas Muhammadiyah Ahmad Dahlan Palembang.

DATA AVAILABILITY STATEMENT

The utilized data to contribute to this investigation are available from the corresponding author upon reasonable request.

DISCLOSURE STATEMENT

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors. The data is the result of the author's research and has never been published in other journals.

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