The Effect of Sample Volume Variation on Blood Glucose Measurements Using POCT Devices within the Context of Public Health Services

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Article Information: received in September 2025; approved in November 2025; published in December 2025

ABSTRACT

Blood glucose testing is a crucial step in the detection and monitoring of diabetes mellitus, especially within the context of public health services. The use of Point-of-Care Testing (POCT) devices has become a practical choice due to their speed and ease of use. However, the accuracy of the test results can be influenced by various factors, one of which is the volume of the blood sample utilized. This study aims to evaluate blood glucose measurements using variations in sample volume on a POCT device. The research employed a laboratory experimental design with a within-subject approach. A total of 55 respondents participated in the study. The blood sample volume variations tested were 0.3 μ I, 0.5 μ I, and 0.7 μ I. The measurements were carried out using the Easy Touch POCT device, and the results were compared with a standard laboratory control instrument. The study findings indicate that a blood sample volume of 0.7 μ I produced glucose values most consistent with the standard laboratory results, where, based on SPSS analysis, the p-value was > 0.05 (0.137 > 0.05), indicating no significant difference between the POCT measurements and the standard laboratory instrument at this volume. In contrast, sample volumes of 0.3 μ I and 0.5 μ I showed significant differences compared to the reference values (p < 0.05), which could affect the accuracy of the measurements and potentially impact the accuracy of diagnosis and patient monitoring. Based on these results, it can be concluded that the volume that yields the highest accuracy for glucose testing using the Easy Touch POCT device is 0.7 μ I. This study highlights the importance of standardizing blood sample volume in the use of POCT to enhance the accuracy of test results, particularly in primary healthcare settings such as community screening programs or public health centers (puskesmas).

Keywords: Blood Glucose; Sample Volume; POCT; Public Health; Accuracy

INTRODUCTION

Diabetes Melitus (DM) constitutes a major global and national public health crisis. In Indonesia, the rising DM prevalence necessitates effective strategies for early diagnosis and rigorous therapeutic management.¹ In this context, blood glucose measurement is central to disease control efforts. To enhance accessibility and efficiency, Point-of-Care Testing (POCT) technology using portable glucometers has been widely adopted, particularly in primary healthcare facilities (PHC) such as *puskesmas* and during field screening activities. The speed of results, ease of use, and operational capability outside the laboratory make POCT crucial in supporting rapid clinical decision-making ³.

Technological advancements have significantly improved POCT blood glucose meters, including developments like enzyme-free electrochemical sensors that enhance sensitivity and specificity. Additionally, integrating POCT devices with electronic information systems reduces manual entry errors and facilitates real-time data management.

Point-of-Care Testing (POCT) is clinical laboratory testing conducted close to the site of patient care where care or treatment is provided. POCT provides rapid turnaround of test results with the potential to generate a result quickly so that appropriate treatment can be implemented, leading to improved clinical or economic outcomes compared to laboratory testing ⁴.

Despite the convenience they offer, the reliability of POCT results is often vulnerable to variations in the pre-analytical phase. One frequently overlooked, yet significantly impactful, pre-analytical factor is the volume of capillary blood sample applied to the test strip. Scientific literature indicates that insufficient blood volume (hypovolume) or, conversely, excessive volume (hypervolume) can interfere with the electrochemical reaction on the glucometer strip, consequently leading to inaccurate readings ^{3 5}Freckmann et al. (2013) ⁶ explicitly documented that even minor deviations from the required sample volume can trigger error messages or, more alarmingly, result in a significant increase in the Mean Absolute Relative Difference (MARD), thereby pushing the results outside the acceptable accuracy limits mandated by international standards like ISO 15197:2013 ⁷.

Recent investigations further highlight this concern. For instance, Mader et al. (2024) emphasized that risk management in POCT glucose monitoring must address pre-analytical variables such as sample sufficiency and operator handling, aligning with the updated ISO 15189:2022 framework for clinical laboratories. Similarly, in their post-market evaluation of four commercial glucometers found that real-world analytical performance may deviate from manufacturer claims, underscoring the need for continuous performance verification in actual

healthcare settings.

While the theoretical foundation regarding the effect of sample volume is established, a fundamental research gap exists within the context of POCT implementation in public healthcare. The majority of existing studies were conducted under highly controlled laboratory conditions, which fail to replicate the realistic challenges and sample volume variations encountered in field settings ⁹. The context of primary healthcare (PHC) in Indonesia presents unique challenges that exacerbate the sample volume issue. In *puskesmas* or during mass screenings, factors such as high time pressure, varied staff training, and suboptimal environmental conditions often lead to non-standardized capillary blood sampling procedures, making it difficult to ensure the correct blood volume is applied to the strip ³.

Furthermore, local studies ¹⁰ have frequently shown a systematic difference in results, with POCT readings tending to be lower compared to standard laboratory methods such as GOD-PAP. A strong hypothesis suggests that suboptimal sample volume in the field may be a major contributor to this systematic bias. This aligns with findings by ¹¹ and ⁸, who observed that small variations in applied sample volume could produce measurable bias and imprecision beyond ISO-accepted thresholds.

Additionally, comparative research between venous and capillary samples indicates that even small variations in sample characteristics may result in measurable though not always clinically significant differences in glucose readings ¹². This further supports the notion that capillary sample handling—including volume consistency—is critical in maintaining result accuracy.

Consequently, this research is highly essential to bridge the existing gap. The present study aims to empirically and quantitatively investigate the impact of blood sample volume variation on POCT glucometers, utilizing a sample volume range that is based on both the device's manual specifications and realistic observations of field practice—encompassing common *hypovolume* (below minimum) and *hypervolume* (above standard) conditions. This approach follows recommendations from contemporary quality management and risk control framework ^{9,13}

The study aims to determine the extent to which glucose measurement results differ (in terms of bias and imprecision) when the sample volume deviates from the required volume, and to assess the clinical implications for the overall reliability of POCT devices within the Indonesian public healthcare setting. The findings are expected to provide a strong evidence base for formulating recommendations regarding the standardization of sampling procedures, the revision of training guidelines for healthcare personnel, and the establishment of quality control policies that are adaptive to the unique challenges at the primary healthcare level in Indonesia.

MATERIAL AND METHODS

This study employed a laboratory experimental design with a within-subject approach, aiming to determine the optimal blood sample volume for glucose measurement using the EasyTouch Point-of-Care Testing (POCT) device. The research was conducted at the Laboratory of the Department of Medical Laboratory Technology, Poltekkes Kemenkes Ternate, after obtaining ethical approval from the Health Research Ethics Committee of Poltekkes Kemenkes Ternate under approval number UM.02.03/6/600/2025. A total of 55 respondents, residing in the working area of Kalumata Health Center, Ternate City, were included in this study, selected through purposive sampling. All participants met the inclusion criteria, namely being diagnosed with type 2 diabetes mellitus, having fasted for at least 8 hours prior to testing, and providing written informed consent to participate in the study.

Each respondent underwent blood glucose measurement using the EasyTouch Point-of-Care Testing (POCT) device. Three different blood sample volumes were applied: $0.3~\mu L$, $0.5~\mu L$, and $0.7~\mu L$. The selection of these volumes was based on both the manufacturer's recommendation and practical considerations: $0.5~\mu L$ represents the minimum volume recommended by the EasyTouch 14 , $0.3~\mu L$ was chosen to simulate hypovolume conditions that may occur in real-world capillary blood sampling, and $0.7~\mu L$ was selected to evaluate hypervolume conditions, reflecting possible over-application during field practice. This range allows the study to assess the effect of both insufficient and excessive sample volumes on the accuracy and precision of POCT glucose measurements.

In addition, a control measurement was performed using the Microlab 300 analyzer with a $10~\mu L$ sample volume as the laboratory reference standard. The Microlab 300 was validated according to the manufacturer's specifications and routinely calibrated to ensure measurement accuracy by using control reagents, serving as a comparison for POCT measurements. Meanwhile, the POCT device was checked before use by inserting the device chip prior to operation.

The venous blood collection procedure was carried out in accordance with the Clinical and Laboratory Standards Institute (CLSI) GP42-A6 guidelines, as follows:

- 1. The respondent was asked to sit in a comfortable position with the arm relaxed.
- 2. The researcher washed their hands with soap and running water, then dried them with a lint-free tissue.
- 3. All venous blood collection equipment was prepared aseptically, including a vacutainer, disposable needle, tourniquet, 70% alcohol swab, gray-top tube, adhesive bandage, and safety box.

- 4. The tourniquet was applied approximately 7–10 cm above the elbow.
- 5. The antecubital area (median cubital vein) was cleaned using a 70% alcohol swab with a circular motion from the center outward and allowed to air-dry.
- 6. A sterile needle was inserted into the vein at an angle of approximately 15–30°, with the bevel facing upward.
- 7. A total of 3 mL of blood was collected.
- 8. The tourniquet was released before withdrawing the needle to prevent hemolysis.
- 9. The needle was carefully withdrawn, and the puncture site was immediately pressed with a sterile dry cotton swab until bleeding stopped.
- 10. The collected blood was transferred into a gray-top tube and labeled with the respondent's identification information.
- 11. After bleeding stopped, the cotton was replaced with an adhesive bandage.
- 12. All disposable materials were discarded into a sharps container according to laboratory safety procedures. ¹⁵

The sample volume adjustment was performed carefully as follows:

- 1. Blood volumes of 0.3 μ L, 0.5 μ L, and 0.7 μ L were measured using a calibrated micropipette (Eppendorf, 0.1–2 μ L capacity) to ensure volume precision in accordance with the study design.
- 2. Each volume was applied directly onto the EasyTouch test strip within five seconds of blood collection to prevent hemolysis or sample drying.
- 3. After each measurement, the test strip and lancet were disposed of in a biohazard sharps container following laboratory safety procedures.

All measurements were conducted sequentially, with an interval of approximately two minutes between each test to maintain the physiological stability of capillary glucose levels and to minimize time-related glucose decline. The order of volume testing was randomized for each participant to minimize systematic bias.

To ensure the validity of the results, testing was performed at the same time in the morning for all participants. ¹⁶
Statistical analysis was performed using SPSS software version 21. Wilcoxon was conducted to assess whether significant differences existed between the different sample volume condition ¹⁷

RESULT

A study on the variation of blood sample volume in glucose measurement using POCT devices in diabetes mellitus patients was conducted on 55 respondents in the Kalumata Public Health Center Work Area. Each sample was divided into three different volumes, namely 0.3 microliters, 0.5 microliters, and 0.7 microliters. The median glucose levels for the sample volumes were 218 mg/dL for 0.3 microliters, 201 mg/dL for 0.5 microliters, 265 mg/dL for 0.7 microliters, and 307 mg/dL for the control.

Table 1. Distribution of Respondents Based on Blood Glucose Measurement Results Using POCT (0.3 μ L, 0.5 μ L, 0.7 μ L) and Control Using Microlab 300

Reference Values for Random Blood Glucose Test	Glucose Testing Using POCT (mg/dL)					Control Clucose Measurement Using Microlab 300		
	0,3 μl		0,5 μl		0,7 μl			%
_ _	n	%	n	%	n	%	n	70
Low (<70)	0	0,00%	0	0,00%	0	0,00%	0	0,00%
Normal (70-180)	27	49,09%	27	49,09%	16	29,09%	14	25,46%
High (>180)	28	50,91%	28	50,91%	39	70,91%	41	75,54%
Total	55	100%	55	100%	55	100%	55	100%

In Table 1, at sample volumes of $0.3~\mu L$ and $0.5~\mu L$, the distribution of blood glucose measurement results showed the same percentage, with 27 samples (49.09%) falling within the normal category (70–180 mg/dL), and 28 samples (50.91%) in the high category (>180 mg/dL). Meanwhile, at a volume of $0.7~\mu L$, there was an increase in the number of samples in the normal category to 16 samples (49.09%) and a decrease in the high category to 39 samples (50.91%). These results indicate that using a sample volume of $0.7~\mu L$ produces a slightly more

balanced distribution between the normal and high categories compared to the previous two volumes. Furthermore, the control measurements using the Microlab 300 device showed that 14 samples (25.46%) were in the normal category and 41 samples (74.54%) were in the high category, with no samples falling into the low category.

Analysis of differences in glucose levels between control values and POCT measurements at various sample volumes was performed using the Wilcoxon Signed-Rank test. Because three pairwise comparisons were performed, the significance level was adjusted using the Bonferroni correction ($\alpha = 0.05/3 = 0.017$) to control for type I error ^{18,19}

Table 2. The results of the Wilcoxon test

The Result		Sig
0,3 Mikroliter		0,005
0,5 Mikroliter	Microlab 300	0,001
0,7 Mikroliter		0,137

Based on Table 2, the results of the non-parametric Mann-Whitney test showed that the significance values (Sig.) for the 0.3 microliter and 0.5 microliter sample volumes were 0.005 and 0.001, respectively, both of which are lower than the predetermined significance threshold ($\alpha=0.05$). This indicates that there is a statistically significant difference between the blood glucose measurements obtained using POCT and the control results obtained using the Microlab 300 device for both sample volumes. In contrast, the significance value for the 0.7 microliter sample volume was 0.137, which is greater than $\alpha=0.05$, indicating no statistically significant difference between the POCT results and the control. Therefore, it can be concluded that significant differences were only observed at the 0.3 μ L and 0.5 μ L volumes, while the 0.7 μ L volume showed consistency with the standard laboratory control measurements.

DISCUSSION

The results of this study indicate a significant difference in blood glucose measurements obtained using the POCT device at sample volumes of 0.3 μ L and 0.5 μ L compared to the laboratory control test (Microlab 300), whereas no statistically significant difference was observed at a volume of 0.7 μ L. These findings are consistent with international literature, which emphasizes that sample volume and pre-analytical factors are critical determinants of POCT accuracy [20,21]. This is also in line with the EasyTouch device manual, which recommends a minimum sample volume of greater than 0.5 μ L.

Several studies have reported that although POCT offers advantages such as rapid testing time, portability, and minimal blood volume requirements, these devices are also highly dependent on the accuracy of sample collection and handling procedures. Errors in the process, such as insufficient sample volume applied to the test strip or delayed measurement, can lead to significant bias compared to laboratory methods using instruments like the Microlab 300 20,21

More specifically, comparative studies have shown that although POCT results correlate well with the GOD-PAP method, there is a bias, particularly at high or low glucose levels 22 . In this study, the significant differences observed at sample volumes of 0.3 μ L and 0.5 μ L suggest that smaller volumes may increase bias because the sample is insufficient to fully saturate the test strip, resulting in suboptimal enzymatic reactions in the measurement area. In addition, a sample volume that is too small can lead to uneven blood distribution within the reaction chamber of the strip, preventing the electrochemical reaction between the enzyme–mediator system and glucose from occurring optimally, this condition is consistent with the literature, which states that incomplete filling of the reaction chamber due to insufficient sample volume can result in lower-than-expected electrochemical current, thereby causing biased glucose readings 23 Furthermore, the literature indicates that hematocrit levels can also affect POCT results, causing glucose readings at certain volumes to be distorted due to variations in hematocrit 24,25

The absence of a significant difference at a sample volume of $0.7~\mu L$ indicates that increasing the sample volume can improve agreement with the laboratory control, likely because the test strip becomes fully saturated, resulting in a more stable sensor reaction. 21 This improved accuracy at $0.7~\mu L$ may also be explained by mechanistic factors at the electrochemical level. A larger sample volume ensures complete wetting of the reaction chamber, allowing the enzyme layer and mediator to dissolve uniformly and enabling more consistent electron transfer at the electrode surface. Adequate sample volume also minimizes localized depletion of glucose during the reaction, ensuring that the redox process proceeds at a steady rate and generates a stable current signal.

Additionally, a fully saturated chamber reduces the influence of capillary flow variability and enhances the diffusion of glucose toward the reactive sites, thereby improving measurement precision.

It is important to note that the EasyTouch device uses the Glucose Oxidase (GOx) method. Sugar in the blood sample reacts with the enzyme layer and the mediator on the test strip, generating an electrical current that triggers a chemical reaction. This reaction is measured by the meter and displayed as your glucose reading. This enzymatic-electrochemical principle explains why proper sample volume and strip saturation are critical for accurate measurements ¹⁴

The results of this study cannot be directly generalized to all POCT brands, as each POCT device has different strip designs and enzyme sensitivities ^{22,26}. Therefore, further research using various POCT brands and models is needed to ensure that the pattern of accuracy dependence on sample volume is consistent.

This study has several limitations. First, only one type/brand of POCT device was used, so the results cannot be generalized to all POCT devices. Second, the sample size was limited, meaning that biological variations among individuals may not have been fully represented. Third, this study did not directly evaluate the influence of hematocrit, even though hematocrit is known to affect glucose measurement results. Fourth, although our respondents had diabetes mellitus (DM), they were in a non-fasting state at the time of sample collection. Further research with a larger sample size, different POCT brands, and control of pre-analytical factors is recommended to strengthen the findings and the external validity of the study.

The significance of this research finding in real-world practice is to reiterate that adherence to an adequate sample volume (a minimum of 0.7 μ L for EasyTouch) is a crucial pre-analytical factor determining the accuracy of blood glucose POCT. Failure to achieve the minimum volume, as occurred at 0.3 μ L and 0.5 μ L, results in biased and potentially lower readings, which is clinically very dangerous as it can lead to incorrect insulin dosing decisions or inaccurate assessment of diabetes status. Therefore, this finding necessitates the standardization of procedures and strict training for healthcare workers (especially in primary healthcare settings like *puskesmas*) and self-monitoring patients, to ensure accurate measurement results, thereby minimizing the risk of dosage error and ensuring patient safetyin diabetes management.

CONCLUSION AND RECOMMEDATIONS

This study concludes that variations in blood sample volume significantly influence the accuracy of blood glucose measurements using Point-of-Care Testing (POCT) devices. The results demonstrated statistically significant differences between POCT measurements and laboratory control values (Microlab 300) at sample volumes of 0.3 μ L and 0.5 μ L, indicating that these lower volumes may yield less reliable results. In contrast, the 0.7 μ L sample volume showed no statistically significant difference from the control, suggesting that it provides a more accurate and consistent reading that aligns better with standard laboratory methods. These findings emphasize the importance of adhering to the recommended sample volume range in POCT procedures to ensure clinically valid and reliable results. In the context of public healthcare services such as community health centers (puskesmas), where POCT is frequently used for screening and monitoring, it is necessary to standardize sample volume collection protocols and provide training for healthcare workers in sample collection, as these are crucial steps to maintain diagnostic accuracy and improve patient care outcomes. For future researchers, it is recommended to conduct studies using different brands of POCT devices.

CONFLICT OF INTEREST

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

ACKNOWLEDMENTS

The author would like to express his gratitude to the Director of the Ternate Ministry of Health Polytechnic and all the academic community.

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