

Blood Collection Tubes Utilized for Fasting Blood Sugar Measurement: A Source of Variation in Clinical Laboratory Test Results

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ABSTRACT

Background. Diabetes mellitus (DM) remains a major global health burden, with increasing prevalence in developing countries such as the Philippines. Accuracy of glucose measurement is vital for diagnosis and management; however, preanalytical variables, particularly glycolysis from varying collection tubes, temperature and time interval can significantly affect test results. Despite guideline recommendations favoring plasma, serum is still commonly used in local clinical practice.

Objective. This study evaluated the effectiveness of different commercially available blood collection tubes in preserving glucose stability by minimizing pre-analytical glycolysis.

Methodology. A cross-sectional observational and quasi-experimental study was conducted among 40 healthy adult participants (18–59 years) from a tertiary institution in Quezon City, Philippines. A total of 160 samples were collected using four tube types (one plasma and three serum tubes). Samples were analyzed at varying time intervals (0–180 minutes) and storage conditions (room temperature and 4°C). Glucose levels were measured using the glucose oxidase method. Statistical analysis included Shapiro–Wilk, Kruskal–Wallis H-test, Welch's t-test, and Dwass–Steel–Critchlow–Fligner post hoc comparisons at a 1% significance level.

Results. Glucose concentrations differed significantly across tube types ($p < 0.001$) and time intervals ($p < 0.001$), but not by storage temperature ($p = 0.023$). Plasma samples demonstrated significantly higher glucose levels than serum samples, with all serum tubes showing a consistent negative bias relative to the fluoride-containing plasma tube. Although differences remained within the ± 6 mg/dL CLIA allowable total error, clinically relevant deviations were observed near diagnostic thresholds. Glucose stability significantly declined beyond 60 minutes, indicating ongoing glycolysis despite sample separation.

Conclusion. Blood collection tube type and delayed processing significantly influence glucose measurements. While acceptable within analytical limits, systematic biases may affect clinical interpretation. Standardization of blood collection practices and stricter pre-analytical protocols are essential to improve diagnostic accuracy for diabetes in the Philippines.

Key words: blood collection tubes, fasting blood sugar (FBS), clinical laboratory science

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INTRODUCTION

Diabetes mellitus (DM) is a chronic disease described to be a group of metabolic diseases characterized by abnormally high glucose levels that result from defects in insulin secretion, insulin action or both.^{1,2} The global prevalence of DM significantly increased over the last decade.³ More so, it has a major impact on third-world countries, particularly the Philippines, with a projected 7.5 million cases by 2045. In 2021, there has been 4.3 million Filipinos with DM, while 2.8 million remained undiagnosed.⁴ There are four clinical types of Diabetes mellitus described according to etiology, Type 1 DM (formerly insulin dependent diabetes mellitus or juvenile diabetes mellitus); results from autoimmune beta-cell destruction, leading to absolute insulin deficiency, Type 2 DM (formerly non-insulin dependent diabetes mellitus or adult-onset DM); results from a progressive insulin secretory defect in the background of insulin resistance, Gestational diabetes



mellitus (GDM): diabetes first diagnosed during pregnancy, and Secondary diabetes: e.g., genetic defects in beta cell function or insulin action, diabetes of the exocrine pancreas (pancreatitis, cystic fibrosis), drug- or chemical-induced diabetes (such as from the treatment of AIDS, after organ transplantation, glucocorticoids), other endocrine diseases (Cushing's syndrome, hyperthyroidism).^{5,6}

Glucose measurement, specifically the fasting blood sugar (FBS) is one of the most commonly done tests in the clinical laboratory. More so, the accurate glucose measurement is key in the diagnosis and management of diabetes patients because this serves as a screening test in detecting hyperglycemia.⁷⁻⁹ FBS levels of patients with DM are characterized to be >126 mg/dL with a threshold level of >180 mg/dL where insulin therapy should be initiated.⁵ Knowing the importance of accuracy in the measurement of blood glucose, the most suitable collection tube for blood glucose determination is still an issue. Previous studies show that the common tube still lacks the ability to immediately prevent clinically significant glycolysis, a process due to the consumption of glucose by the blood cells (RBC, WBC, and platelets), even in so-called gold standard tubes containing sodium fluoride with potassium oxalate. It is also compared to serum tube and citrated tubes which were recommended to be considered for glucose measurement.¹⁰ While a 2019 study by Bonetti, G. et al. shows that tubes with sodium fluoride/citrate buffer liquid mixture and lithium-heparin tube have equal results in avoiding glycolysis completely and within 4 hours.¹¹ Currently, in the Philippines the majority of clinical laboratories are still using serum rather than plasma as the sample for FBS testing.

This practice is contrary to the Philippines Diabetes Clinical Practice Guidelines (CPG) recommendation, which is to utilize plasma for the said testing. As to the advantage of plasma samples, it allows immediate sample tube centrifugation without waiting for the blood to clot.¹² In this regard, failures in the proper specimen handling and collection is considered as one of the possible sources of variability in the medical laboratory test results as part of the pre-analytical phase errors with the highest percentage of 46% up to 77%, compared to the analytical and post-analytical phases.^{13,14}

Moreso, since accurate measurements of blood glucose levels are crucial for the diagnosis of diabetes, proper blood collection tubes should be used to preserve the quality of the samples. In this study, the researcher evaluated the capability of different commercially available serum tubes in preserving the quality of the blood samples by preventing clinically significant glycolysis.

METHODOLOGY

Research design

The research design used in this research is cross-sectional observational and quasi-experimental design, as it attempted to compare the glucose measurements from different collection tubes commonly used in the clinical laboratory. This was the design used because the aim of this research is to observe whether there is relatedness between the glucose levels and the collection tubes used temperature variation and interval in its measurement.

Only observation of data was used, and no intervention were used to obtain the results.

Study population, sample and sampling technique

The intended study population for this research are teaching and non-teaching personnel of tertiary institution in Quezon City, Philippines. The age range for that participants were from 18 to 59 years old. The healthy volunteers were recruited from March 2022 to April 2022. Nonprobability sampling was used as the sampling technique for this research. More so, the subjects were required to fast for 8-12 hours. Subjects who exceeded the said fasting requirement were rejected from the study. These patient preparations, data gathering procedures and blood collection procedures were thoroughly discussed to the participants through an orientation prior to specimen collection. The orientation also discussed the contents of the informed consent form, the participants' rights in the study, how their anonymity and confidentiality will be ensured, and the potential risks and mitigations were all given emphasis. Lastly, the orientation was done personally or through an online platform. Once consents were given and the orientation successfully done blood collection started the next day. The samples that were used in this study were the serum and plasma samples collected from the teaching and non-teaching personnel.

A total of one hundred-sixty (160) samples from 40 subjects were analyzed in this study. In addition, any information about the participants that were collected were kept confidential, which only the researcher can access. The information of the patients, such as name, age and birthdate gathered during specimen collection were kept confidential with the use of patient accession number and so their names will not be divulged. Meanwhile, information about the participants were kept in a file with a lock key, for electronic data it was kept in a folder with password and authentication, and it will not be shared with anyone, especially those not related to the research.

The samples from each subject were aliquoted, for each blood collection tube, two tubes were prepared and labelled according to the accession number of the specimens. The serum and plasma were separated from their respective tubes and divided to each tube equally, one to be stored at room temperature, 26.0 °C (range 23.6–28.6 °C) and at refrigerated temperature, 4.0 °C. It was then measured at varying time intervals, immediately after centrifugation, after 30 minutes, after 60 minutes, 120 minutes and 180 minutes.

Research instrument

An informed consent form and questionnaire were given to the teaching and non-teaching personnel of tertiary institution in Quezon City, Philippines who participated in the study. The questionnaire contained questions regarding their relevant clinical history, work experience and environment. These questions focused on recent hospitalization, medication and diet and lifestyle.¹¹ The equipment used for the collection of samples were evacuated tubes and needles, tourniquet, alcohol pads, gauze pad, and sharps container. The materials used for the glucose measurement are centrifuge, glucose oxidase reagent (Randox), and the Biosystems A15 machine.

Blood collection

Blood samples were collected by a single experienced phlebotomist to minimize venipuncture bias and were carried out between 7 am and 11 am to limit the effects of diurnal and circadian rhythm.¹⁵⁻¹⁷ Samples were collected through an evacuated tube system to minimize contamination and to allow the collection of multiple blood collection tubes from the subjects.¹⁸⁻²⁰ The room temperature during blood collection was 26.0 °C (range 23.6–28.6 °C). Visibly lipemic, icteric or hemolyzed samples were not included in the study.^{19,21-23} The blood collection tubes that used were the plasma tubes from BD Vacutainer sodium fluoride/sodium EDTA 13 x 75 mm (P1 Tube), 2 ml and the serum tubes used were BD Vacutainer plain serum tube 13 x 75 mm (S1 Tube), 4 ml, BD Vacutainer serum tube with clot activator 13 x 75 mm, 4 ml (S2 Tube), and BD Vacutainer serum separator tube (SST) 13 x 100 mm (S3 Tube), 4 ml.²⁴ Blood collection procedures were carried out following the Clinical and Laboratory Standards Institute (CLSI) Guidelines GP 41. A total of 160 specimens were drawn. Forty (40) tubes for each blood collection tube mentioned above. More so, the samples were collected at the phlebotomy room of the College of Medical Technology Diagnostic laboratory. Therefore, there were no delays in transportation and no effect on environmental temperature.

Specimen processing and aliquot preparation

After collection, serum tubes were allowed to clot for 60 minutes for S1, 20 minutes for S2 and S3;¹⁶ and then all four tubes, including P1 were centrifuged at 1600 × g for 10 minutes using the DSC1512T of Digisystems centrifuge. The aliquot preparation was carried out immediately after centrifugation. Two tubes were labelled according to the accession number of the specimens. The serum and plasma were separated from their respective tubes and aliquots of the samples were prepared, containing approximately 1.0 mL aliquots of plasma and serum. For each blood collection tube, two tubes were prepared and labelled according to the accession number of the specimens, one to be stored at room temperature, 26.0 °C (range 23.6–28.6 °C) and at refrigerated temperature, 4.0 °C. In addition, prior to the measurement of samples visibly lipemic, hemolyzed, and icteric was not included in the study

Glucose measurement

The glucose concentration was determined spectrophotometrically using Biosystem A15 autoanalyzer (Biosystems Inc.) by a glucose oxidase-peroxidase method (Randox).²⁵ Prior to the measurement, calibration procedures were done. As for the quality control procedures, the researcher ran a two-level of quality control material at the beginning of each working day before starting any measurement. This was conducted by the researcher who is also qualified technical personnel and results were properly documented. All the specimens from the participants were analyzed using the same lot of reagents, eliminating any lot-to-lot variability in the results. The researcher performed the measurements of all study specimens. The glucose concentration was measured immediately after centrifugation x and with intervals of 30 minutes, 60 minutes, 120 minutes, 180 minutes.^{26,27}

Inclusion and exclusion criteria

Both teaching and non-teaching personnel of tertiary institution in Quezon City, Philippines who were willing to participate in this study were enrolled. Individuals with comorbidities such as chronic liver disease, malignancy, autoimmune disease, and pregnant women were excluded. In addition, this study did not include individuals with prediabetes, established diabetes, or gestational diabetes mellitus; therefore, the diagnostic impact of the observed plasma–serum and tube-related differences across all glycemic categories could not be fully assessed.

Statistical treatment of data

For the statistical treatment of the data, prior to conducting the analysis, the test of normality was done using Shapiro-Wilk statistics which showed that the assumption of normality was violated, $W = 0.982$, $p = <0.001$. Thus, the researcher used the non-parametric statistical tools, Kruskal-Wallis H-Test as an alternative to one-way ANOVA and Dwass-Steel-Critchlow-Fligner (DSCF) pairwise comparisons for the post-hoc analysis. All of which have a 1% level of significance. These were used in determining if there were significant differences among the glucose measurement using varying blood collection tube, storage temperature and time interval prior to measurement.^{12,16}

Ethical clearance

The study was carried out in accordance with the ethical standards set by the Trinity University of Asia—Institutional Ethics Review Committee (TUA—IERC). Most importantly, the research protocol and written informed consent utilized in this study were reviewed and approved by TUA—IERC with a research protocol code number, 2022-009-Ganding-GS-FBS-v2.

RESULTS AND DISCUSSION

A total of 40 subjects participated and provided blood samples for the four different blood collection tubes: BD Vacutainer sodium fluoride/sodium EDTA 13 x 75 mm (P1 Tube), BD Vacutainer plain serum tube 13 x 75 mm (S1 Tube), BD Vacutainer serum tube with clot activator 13 x 75 mm, 4 ml (S2 Tube), and BD Vacutainer serum separator tube (SST) 13 x 100 mm (S3 Tube) for an overall total of 400 blood samples processed during the experiment. After specimen collection, the tubes were inverted following the manufacturer's recommendation then were allowed to clot serum tubes were allowed to clot according to the for 60 minutes for S1, 20 minutes for S2 and S3;¹⁶ and then all four tubes, including P1 will be centrifuged at 1600 × g for 10 min using the DSC1512T of Digisystems centrifuge. The aliquot preparation was carried out immediately after centrifugation. Two tubes were labelled according to the accession number of the specimens. The serum and plasma were separated from their respective tubes and divided to each tubes equally. After the initial glucose measurement, tubes were stored at room temperature (26.0 °C) or at refrigerated temperature (4.0 °C) and measured repeatedly after 30, 60, 120, and 180 minutes.

Since the glucose concentration from the four different tubes, differs significantly at 1% level of significance, $H(3) = 25.3$, $p = <0.001$ as shown in Table 4 a post hoc analysis using DSCF for pairwise comparison was done to specifically

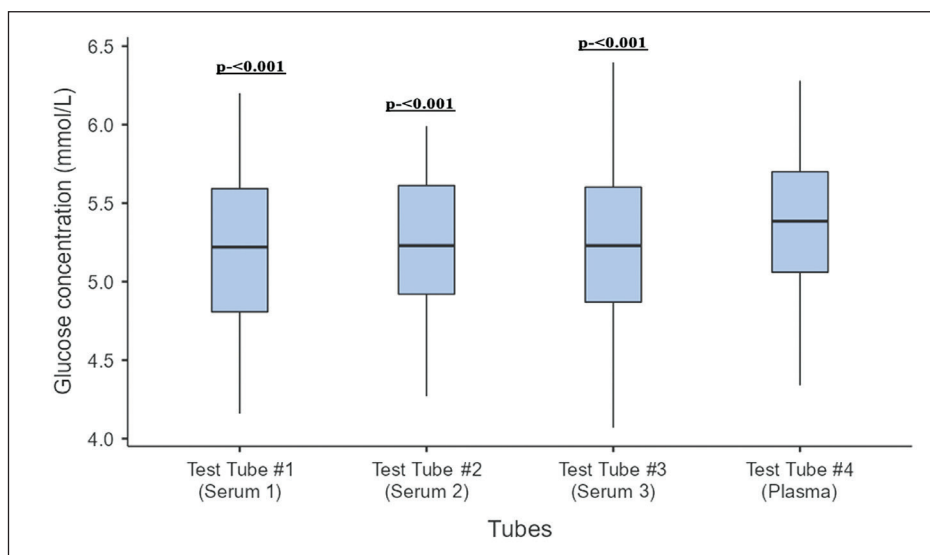


Figure 1. Difference of glucose measurement with varying collection tubes.

Table 1. Post-hoc analysis using Dwass-Steel-Critchlow-Fligner pairwise comparisons on the difference of glucose measurement with varying collection tubes

Pairwise comparisons – Glucose concentration (mmol/L)		W	p
Test Tube #1 (Serum 1)	Test Tube #2 (Serum 2)	2.022	0.481
Test Tube #1 (Serum 1)	Test Tube #3 (Serum 3)	0.674	0.964
Test Tube #1 (Serum 1)	Test Tube #4 (Plasma)	6.337	<0.001
Test Tube #2 (Serum 2)	Test Tube #3 (Serum 3)	-1.429	0.744
Test Tube #2 (Serum 2)	Test Tube #4 (Plasma)	4.598	0.006
Test Tube #3 (Serum 3)	Test Tube #4 (Plasma)	5.754	<0.001

identify the tubes that had a significant difference in their glucose concentration (mmol/L). As shown in Tables 5 and 7, complemented by Figure 5, Test tube 1- Serum 1 (MD = 5.22); Test Tube #2 - Serum 2 (MD = 5.23); and Test Tube #3 - Serum 3 (MD = 5.23) are significantly different from the Test Tube #4 Plasma (MD = 5.38), $W = 6.337, p = <0.001, W = 4.598, p = 0.006, W = 5.754, p = <0.001$, respectively.

These findings agree with the conclusion of Pant et al., that the use of different tubes for glucose measurement yields varying results. This is contrary with the findings of Winter et al., who mentioned that glucose concentration measured from serum tubes, specifically those with thixotropic gel (S3) would remain unchanged. However, the findings in this study further agrees with Bhatt et al., that the glucose concentration measured from the plasma collected using the gray top tube containing NaF/Na EDTA tube has higher glucose concentration compared to serum tubes. This can be explained and attributed with the additive found in P1, the sodium fluoride (NaF) which is an antiglycolytic agent.²⁸ Fluoride inhibits enolase, an enzyme involved in glycolysis which requires magnesium for its optimal activity.⁷ This is the very reason this tube is also recommended by CLSI and ADA as a gold standard for glucose measurement.

Prior to conducting the analysis, Tables 2 and 3 show the test of normality was examined using Shapiro-Wilk

Table 2. Glucose concentration in varying temperature storage prior to measurement

Shapiro-Wilk	W	p
Glucose concentration (mmol/L)	0.982	<0.001

Note: A low p-value suggests a violation of the assumption of normality

Table 3. Homogeneity of variances test (Levene's)

	F	df	P
Glucose concentration (mmol/L)	0.712	1	0.399

Note: A low p-value suggests a violation of the assumption of equal variances

Table 4. Independent samples t-test

Glucose concentration (mmol/L)	Welch's t		Cohen's d
	Statistic	p	Effect Size
Glucose concentration (mmol/L)	2.28	0.023	0.114

Table 5. Difference of glucose measurement with varying storage temperature

Glucose concentration (mmol/L)	Group	Mean	Median	SD
	Room Temp.		5.28	5.29
Refrigerated Temp.		5.23	5.24	0.449

statistics which showed that the assumption of normality was violated, $W = 0.982, p = <0.001$. Aside from this, test of homogeneity of variance test using Levene's test was utilized and found out that a violation of the assumption of equal variances, $F = 0.712, p = 0.399$ was violated. Thus, Welch-Test was used as an alternative to parametric T-Test for independent samples.

Table 4 shows the result of Welch-Test reveals that the glucose concentration did not differ significantly among the varying temperatures storage prior to measurement at 1% level of significance, $t(1)=2.28, p = 0.023$. There is a moderate effect size in this analysis reflected in Cohen's ($d = 0.114$). In addition, Table 5, complemented

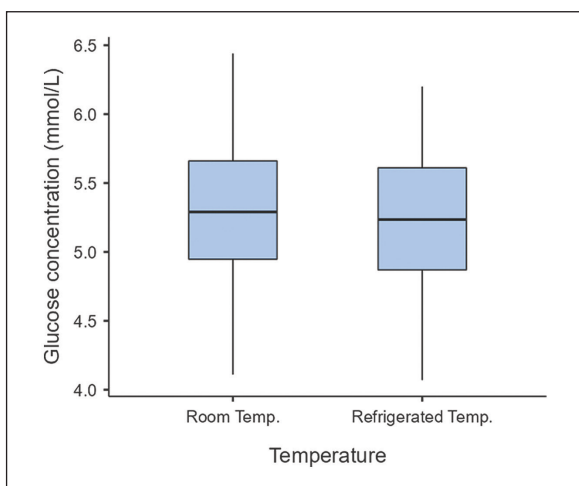


Figure 2. Difference of glucose measurement at varying temperature storage.

by Figure 2, presents the descriptive on the difference of glucose measurement with varying storage temperature.

The findings of this study shows that varying temperature storage prior to measurement is not significant. This suggests that regardless of storage temperature, glucose concentration is unaffected. This is contrary to the findings of Kubihal et al., who mentioned that glucose is only stable at lower temperatures. This may be due to a more robust condition of this study that used a significance level of 1% compared to the other studies that used a 5% level of significance.

Glucose concentration in varying time intervals prior to measurement

Table 6 shows the result of Kruskal-Wallis H-Test reveals that the glucose concentration differs significantly at 1% level of significance among the different time intervals prior to measurement, $H(3)=55.3, p = <0.001$. There is a weak effect size in this analysis ($\epsilon^2 = 0.0346$).

Meanwhile, Table 7 shows the Post hoc analysis using DSCF showed that the glucose concentration (mmol/L) at different time interval specifically after 120 minutes (MD = 5.17); after 180 minutes (MD = 5.17); are significantly different from the glucose concentration measured immediately after centrifugation (MD = 5.46), $W = -7.36, p = <0.001, W = -8.82, p = <0.001$, respectively. In addition, glucose concentration (mmol/L) measured after 120 minutes (MD = 5.17); after 180 minutes (MD = 5.17); are also significantly different from the glucose concentration measured after 60 minutes (MD = 5.36), $W = -75.74, p = <0.001, W = -7.08, p = <0.001$, respectively.

This study suggests that glucose, whether in plasma and serum, is optimally stable only up to one hour. This agrees with study made by Winter et al., who mentioned that delayed specimen processing and measurement beyond one hour should be avoided. As seen in Table 7, complemented by Table 8, there is significant decrease in glucose concentration with the increasing lapse of time, the difference being more with increase in time gap, which also agrees with the conclusions of Bhargava

Table 6. Difference of glucose measurement at varying time intervals prior to measurement

	Kruskal-Wallis	χ^2	df	p	ϵ^2
Glucose concentration (mmol/L)		55.3	4	<0.001	0.0346

Table 7. Post-hoc analysis using Dwass-Steel-Critchlow-Fligner pairwise comparisons on the difference of glucose measurement with varying time intervals prior to measurement

Pairwise comparisons – Glucose concentration (mmol/L)		W	P
Immediately after centrifugation	30 minutes	-4.39	0.016
	60 minutes	-2.03	0.607
	120 minutes	-7.36	<0.001
	180 minutes	-8.82	<0.001
30 minutes	60 minutes	2.52	0.386
	120 minutes	-3.17	0.163
	180 minutes	-4.48	0.013
60 minutes	120 minutes	-5.74	<0.001
	180 minutes	-7.08	<0.001
120 minutes	180 minutes	-1.18	0.921

Table 8. Descriptive on glucose concentration glucose measured at varying time intervals prior to measurement

	Time	Median
Glucose concentration (mmol/L)	Immediately after centrifugation	5.46
	30 minutes	5.32
	60 minutes	5.36
	120 minutes	5.17
	180 minutes	5.17

et al. Currently, the CLSI guidelines would recommend processing of specimens within two hours but based on the results of this study the difference is already significant. This finding aligns with the results of Bhatt et al., The significant decrease may be attributed to the continuous glycolysis due to the presence of red blood cells and white blood cells even after separation of plasma or serum.^{11,29}

Although the decreased in the glucose concentration seen from measurements done after two and three hours are statistically significant, the difference is within the ± 6 mg/dL (0.33 mmol/L), which is within the standard acceptable range as defined by the United States Clinical Laboratory Improvement Amendments (CLIA) guideline.^{12,16,28} However, in the Philippines, there are no guidelines similar to CLIA yet.

Based on the data collected from this study it shows that over all glucose concentration measured using different blood collection tubes, stored at room and refrigerated temperature and are measured at varying time interval are significantly different. While there is no significant difference in the glucose concentration measured with varying temperature storage prior to measurement. It can be noted that glucose concentration varies significantly when stored in room and refrigerated temperatures. S1 has decreased by 4.05% at room temperature and 2.85% at refrigerated temperature; S2 has decreased by

5.78% at room temperature and 7.47% at refrigerated temperature; S3 has decreased by 4.60% at room temperature and 4.18% at refrigerated temperature; and P1, at the end of the observation the median glucose decreased by 5.72% at room temperature and 8.42% at refrigerated temperature. Moreso, the overall glucose concentration from the four different tubes, differs significantly at 1% level of significance, $H(3)=25.3$, $p < 0.001$. Among the three serum tubes, S2-BD Vacutainer serum tube with clot activator has the least Wilcoxon rank sum test statistic of $W = 4.598$.

However, contrary to common notion, at 1% level of significance, glucose concentration did not differ significantly at either room or refrigerated temperature used as storage prior to testing. As recommended and suggested by the researcher, based on this study, glucose, whether in plasma and serum, is optimally stable only up to one hour. Additionally, the red top tube (S3) with a clot activator can be used as an alternative to gray top tube, as it can be centrifuged within 30 minutes and analyzed within an hour. This would optimally prevent significant glycolysis in the absence of a tube containing an antiglycolytic agent.

CONCLUSION

This study demonstrates that pre-analytical variables significantly influence glucose measurement outcomes. The results revealed a statistically significant difference in glucose concentration between plasma and serum, with plasma glucose consistently higher than serum glucose. While the median numerical differences are within the 6mg/dL (0.33 mmol/L) allowable total error defined by CLIA, this systematic negative bias in serum relative to plasma becomes clinically relevant near diagnostic cut-offs such as the ADA fasting glucose threshold of 126 mg/dL. Overall collection conditions, glucose concentrations measured using different blood collection tubes, stored at room or refrigerated temperature, and measured at varying time intervals were significantly different. These findings emphasize the critical role of pre-analytical standardization in ensuring reliable glucose measurement. The implementation of standardized blood collection protocols, including appropriate tube selection and timely processing, is essential to improve diagnostic accuracy and support optimal clinical decision-making for diabetes in the Philippines.

RECOMMENDATIONS

Based on these findings, timely specimen processing should be prioritized, with blood samples centrifuged within 20–30 minutes and analyzed within one hour of collection whenever possible. The use of blood collection tubes containing antiglycolytic agents is strongly recommended to minimize ongoing *in vitro* glycolysis. When serum tubes are used, those with clot activators should be preferred to shorten clotting time and reduce serum contact with blood cells. Clinical laboratories are encouraged to standardize the type of blood collection tubes used for glucose testing to reduce variability in results. Future studies including individuals with prediabetes, diabetes, and gestational diabetes mellitus are warranted to determine how these pre-analytical differences affect diagnosis across all

glycemic categories. Finally, the development and implementation of local laboratory protocols defining acceptable pre-analytical conditions and reference ranges for glucose monitoring are essential to promote consistency, diagnostic accuracy, and improved patient care outcomes.

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