STABILITY OF SERUM GLUCOSE ON BARRIER GEL COMPARED WITH PLAIN TUBES POST CENTRIFUGATION DURING 24 AND 48 HOURS OF STORAGE AT 2-8 °C

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ABSTRACT

Pre-analytical errors represent the primary source of variation in clinical laboratory testing, frequently caused by delayed specimen processing post-centrifugation. Prolonged contact between serum and the erythrocyte clot accelerates in vitro glycolysis, falsely reducing glucose concentrations. This study aimed to determine the stability of serum glucose stored on top of a barrier gel in Serum Separator Tubes compared to non-gel plain tubes after 24 and 48 hours of refrigerated storage at 2–8 °C. A repeated-measures experimental design was conducted using venous blood specimens from 33 healthy volunteers. Blood samples collected into both tube types were centrifuged simultaneously and analyzed immediately, as well as after 24 and 48 hours using the enzymatic glucose oxidase method. Statistical analysis revealed a significant interaction between tube type and storage duration ($p < 0.001$). Serum glucose in Serum Separator Tubes decreased marginally by 0.69% at 24 hours and 1.48% at 48 hours, remaining within acceptable clinical limits. Conversely, glucose in plain tubes decreased by 9.97% at 24 hours and 18.84% at 48 hours, exceeding clinical tolerance thresholds. In conclusion, Serum Separator Tubes preserve serum glucose stability for up to 48 hours without requiring manual aliquoting, whereas non-gel tubes cause substantial time-dependent analytical bias.

KEYWORDS Blood Glucose; Glycolysis; Pre-analytical Phase; Serum Separator Tube; Specimen Handling

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INTRODUCTION

The pre-analytical phase represents the most vulnerable stage in the modern clinical laboratory testing workflow, accounting for approximately 60% to 70% of all diagnostic error incidents [1, 2]. Among routine biochemical blood analytes, glucose is particularly susceptible to pre-analytical variability caused by specimen handling and processing conditions [3]. Physiologically, viable cellular blood components notably erythrocytes and leukocytes continue to consume metabolic energy through in vitro glycolysis following phlebotomy [4]. This ongoing biochemical cascade depletes glucose at an estimated rate of 5% to 7% per hour at controlled room temperature [5].

In daily operations within primary healthcare facilities and referral diagnostic centers, post-centrifugation testing delays are frequently unavoidable due to heavy specimen backlogs, instrument batch testing protocols, and specimen transport logistics [6, 7]. Standard operational guidelines advocate for immediate physical separation (aliquoting) of serum from the underlying cellular clot into secondary tubes to terminate metabolic contact with erythrocytes [8]. However, manual aliquoting is labor-intensive, adds consumable costs for secondary microvials, and elevates the risk of occupational biohazard exposure such as infectious aerosols or biofluid spillage—for laboratory personnel [9, 10].

To address these limitations, innovative vacuum Serum Separator Tubes (SST) containing a thixotropic polymer gel were engineered to form an automated physical barrier between cellular components and the serum phase during centrifugation [11, 12]. Despite this advance, real-world laboratory workflows often bypass secondary aliquoting entirely, leaving centrifuged primary tubes stored directly in refrigerators (2–8 °C) [13]. Although cold storage (2–8 °C) slows down the kinetics of cellular glycolytic enzymes, it does not completely arrest analyte diffusion if physical interphase contact is maintained [14]. While prior studies have addressed glucose stability under routine conditions, a clear evaluation comparing prolonged in-tube gel barriers against plain tubes without gel under delayed refrigerated storage particularly assessing both analytical and clinical acceptability criteria remains critically needed to establish safe specimen hold times.

Therefore, this study aims to evaluate and establish the stability of serum glucose concentrations retained over a thixotropic gel barrier (SST) compared to plain non-gel tubes post-centrifugation during 24 hour and 48-hour storage periods at 2–8 °C, evaluated against both analytical statistical benchmarks and clinical acceptability criteria.

RESEARCH METHOD

Research Design, This study was a true experimental laboratory investigation employing a within-subjects repeated measures design. It evaluated the effects of collection tube types and delayed refrigerated storage durations on serum glucose concentrations. Population, Sample, and Ethical Considerations. The study population comprised healthy adult volunteers. A total of 33 eligible participants (n = 33) were enrolled after voluntarily signing informed consent forms. Apparatus and Materials Specifications. The instruments and consumables utilized in this experiment included:

1. Blood Collection Tubes:
    1) *Tube A*: Serum Separator Tube (SST) containing a thixotropic polymer barrier gel and clot activator (gold/yellow top).
    2) *Tube B*: Plain vacuum tube containing a clot activator without barrier gel (red top).

2. Laboratory Equipment: Standard clinical benchtop centrifuge (set at 3,000 rpm), spectrophotometer/photometer (500 nm wavelength), precision micropipettes (10 µL), laboratory-grade refrigerator (calibrated at 2–8 °C), and parafilm seal wrap.
3. Reagents and Quality Controls: Glucose Oxidase–Peroxidase (GOD-PAP) enzymatic photometric reagent kit and commercial normal-level control sera.

Data Collection and Experimental Procedures

1. Phlebotomy: Venous blood collection from the median cubital vein was conducted adhering strictly to the EFLM-COLABIOCLI phlebotomy recommendations [16]. Tube drawing sequence and tube volume integrity complied with standard color-coding protocols [17]. A total of 6 mL of venous blood was collected from each subject and immediately divided equally (3 mL each) into Tube A and Tube B.
2. Clotting and Centrifugation: Following the Clinical and Laboratory Standards Institute (CLSI) specimen processing guidelines [16], both tubes were allowed to clot completely in an upright position at 20–25 °C for 25 minutes. Both tubes were then simultaneously centrifuged at 3,000 rpm for 10 minutes.
3. Baseline Measurement (T_0): Immediately after centrifugation, a 10 µL aliquot of the uppermost serum layer was aspirated from each tube for baseline glucose determination (A_0 and B_0).
4. Specimen Storage: The remaining serum in the primary tubes was kept intact directly over the barrier gel (Tube A) or the cellular clot (Tube B), tightly sealed with paraffin film to prevent analyte evaporation, and stored in a laboratory refrigerator at 2–8 °C [13,19].
5. Follow-up Measurements (T_24 and T_48): Re-testing was executed at 24 hours (A_24, B_24) and 48 hours (A_48, B_48) of storage. Prior to photometric measurement, tubes were equilibrated at room temperature for 15 minutes.
6. Analytical Determination: Serum glucose concentrations were determined photometrically using the GOD-PAP enzymatic method at $\lambda = 500$ nm [17,20]. Internal analytical quality control was verified using commercial normal-level control serum prior to each analytical run [18, 21].

RESULT AND DISCUSSION

Figure 1. Research Stages Flowchart

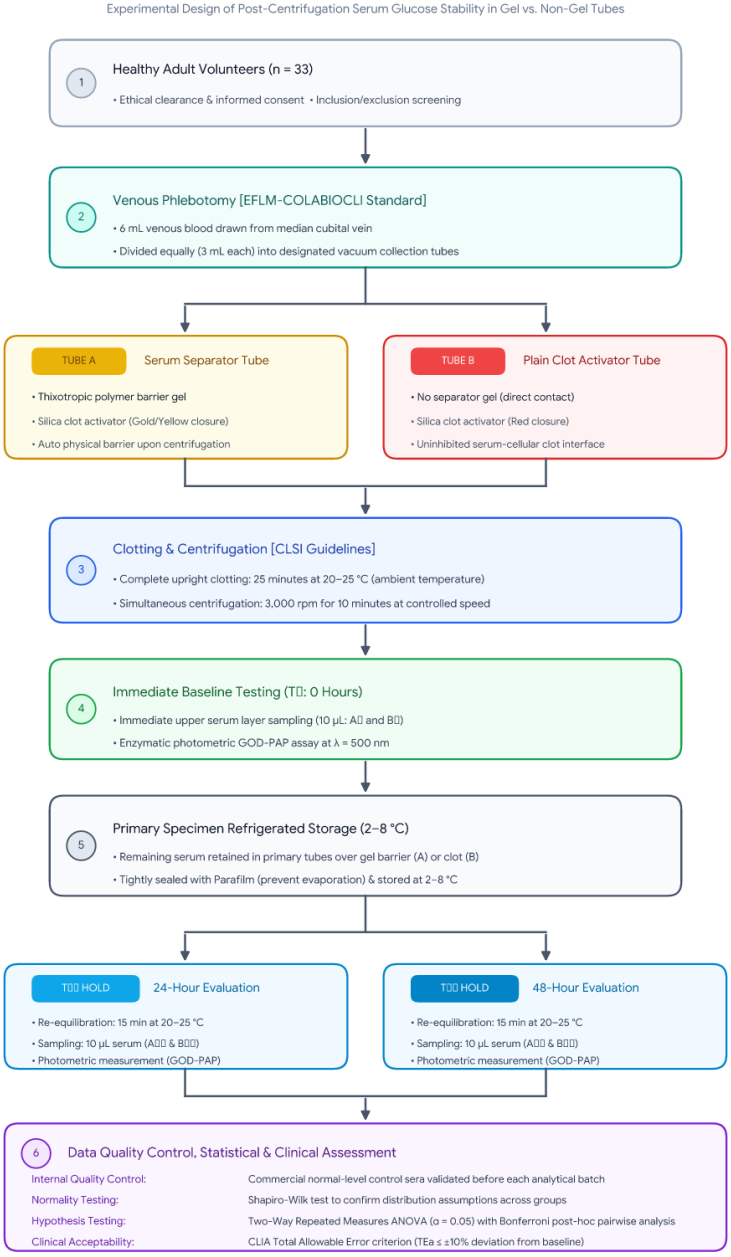


Figure 1. Research Stages Flowchart

Data Analysis

The research variables comprised:

1. Independent Variables: Tube type (SST with thixotropic gel vs. plain tube without gel) and storage duration (0, 24, and 48 hours).
2. Dependent Variable: Serum glucose concentration (mg/dL).

Normality of data distribution was tested using the Shapiro-Wilk test. Differences across tube types and storage intervals were analyzed using Two-Way Repeated Measures

Analysis of Variance (Two-Way RM-ANOVA) at a significance threshold of \alpha = 0.05, followed by Bonferroni post-hoc tests for pairwise comparisons [18,22]. In addition to statistical testing, clinical significance was assessed against the Clinical Laboratory Improvement Amendments (CLIA) regulatory criteria for glucose, defined by a Total Allowable Error (TE a) of ≤±10% [19,23].

The Shapiro-Wilk test confirmed that all dataset subsets met the assumption of normal distribution ($p > 0.05$). The mean serum glucose concentrations in the SST group at 0, 24, and 48 hours were 92.36 mg/dL, 91.73 mg/dL, and 91.00 mg/dL, respectively, representing percentage reductions of 0.69% at 24 hours and 1.48% at 48 hours. In contrast, the plain non-gel tubes demonstrated a marked decline in mean glucose levels from 92.67 mg/dL at baseline (0 hours) to 83.42 mg/dL at 24 hours (a 9.97% decrease) and 75.21 mg/dL at 48 hours (an 18.84% decrease). The numerical summary of analyte stability and clinical acceptability evaluations across both tube types is presented in Table 1.

Table 1. Summary of Serum Glucose Stability and Clinical Acceptability Evaluation (n = 33)

Tube Type	Storage Duration	Mean ± SD (mg/dL)	Difference (Δ) (mg/dL)	Mean Decrease (%)	CLIA TEa Limit (±10%)	Clinical Acceptability Status
SST (Gel)	0 h (Baseline)	92.36 ± 10.10	0.00	0.00%	± 10\%	Acceptable (Reference Value)
	24 h	91.73 ± 9.98	-0.64	0.69%	± 10\%	Clinically Acceptable
	48 h	91.00 ± 10.04	-1.36	1.48%	± 10\%	Clinically Acceptable
Plain (Non-gel)	0 h (Baseline)	92.67 ± 10.15	0.00	0.00%	± 10\%	Acceptable (Reference Value)
	24 h	83.42 ± 9.15	-9.24	9.97%	± 10\%	Marginally Acceptable (Critical Limit)
	48 h	75.21 ± 8.41	-17.45	18.84%	± 10\%	Rejected (Clinically Significant Bias)

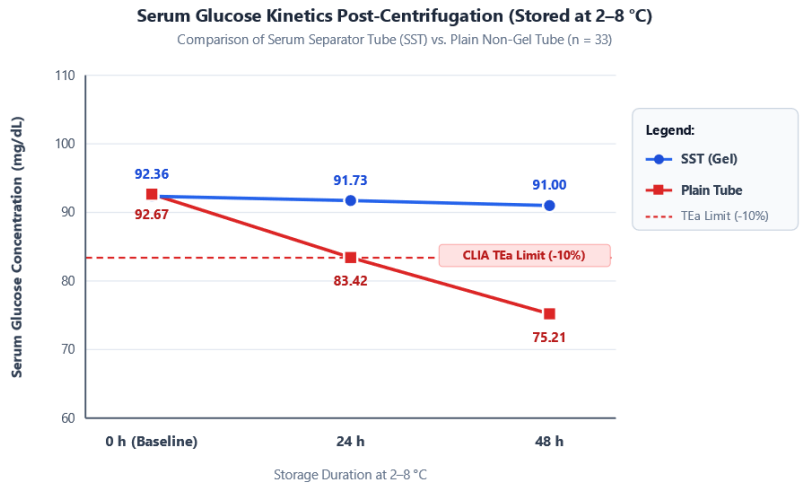


Figure 2. Serum Glucose Kinetics Post-Centrifugation (Stored at 2-8^0 C)

The Two-Way Repeated Measures ANOVA revealed a statistically significant interaction effect between tube type and storage duration on serum glucose concentrations

($F(2,64)=642.880, p<0.001, \eta p^2=0.953$). The kinetics of glucose depletion differed significantly between barrier-gel tubes and non-gel tubes across the prolonged storage intervals. Statistically significant main effects were also observed for tube type ($F(1,32)=582.140, p<0.001, \eta p^2=0.948$) and storage duration ($F(1.684,53.888)=812.450, p<0.001, \eta p^2=0.962$). The complete inferential statistical outcomes are detailed in Table 2.

Table 2. Inferential Results of Two-Way Repeated Measures ANOVA

Source of Variation	Degrees of Freedom (df)	Calculated F-value	Significance (p)	Effect Size (ηp2)	Statistical Conclusion
Tube Type	1	582.140	< 0.001	0.948	Significant (p < 0.05)
Storage Duration	1.684*	812.450	< 0.001	0.962	Significant (p < 0.05)
Interaction (Tube \times Time)	2	642.880	< 0.001	0.953	Significant (p < 0.05)

**Degrees of freedom were corrected using the Greenhouse-Geisser estimate (\epsilon = 0.842) due to a violation of Mauchly's test of sphericity.*

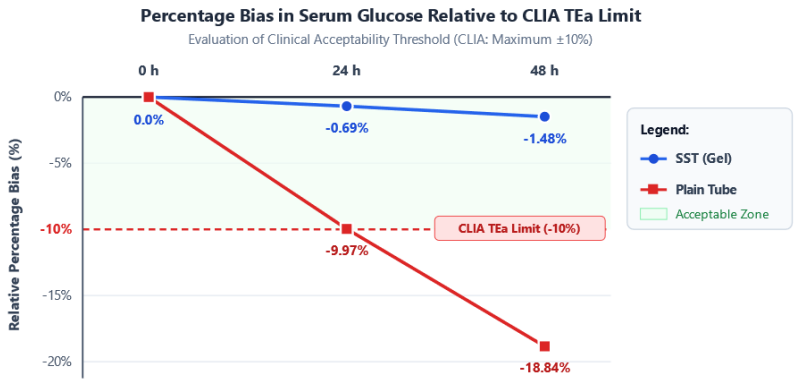


Figure 3. Percentage Bias in Serum Glucose Relative to CLIA TEa Limit

The sustained stability of serum glucose concentrations observed in Serum Separator Tubes (SST) is directly attributable to the physicochemical properties of the thixotropic polymer gel [24]. Formulated with a specific gravity of approximately 1.04 g/cm³ an intermediate density between serum (1.02 g/cm³) and packed cellular elements (1.08–1.09 g/cm³) the gel undergoes reversible liquefaction under centrifugal shear forces and migrates to form an impermeable, solid barrier (2–4 mm in thickness) directly above the cellular clot. This automated mechanical partition effectively eliminates subsequent physical contact between serum and metabolically active blood cells [25].

The minimal decline in glucose levels observed in SST specimens throughout the 48-hour cold storage duration remained well within the regulatory limits established by the Clinical Laboratory Improvement Amendments (CLIA) Total Allowable Error (TE a $\leq \pm 10\%$). This finding is consistent with the evaluative reports of Padoan et al. [26, 27], confirming that a structurally intact gel barrier successfully prevents post-centrifugation molecular diffusion and metabolite exchange during refrigerated preservation. Consequently, primary SST containers maintain analytical and diagnostic integrity without requiring immediate, labor-intensive aliquoting into secondary tubes.

Conversely, the progressive degradation of glucose concentrations in plain tubes without gel demonstrates that refrigerated storage (2–8 °C) alone is insufficient to

completely arrest erythrocyte glycolysis [13, 28]. Although hypothermic conditions suppress the kinetics of key rate-limiting glycolytic enzymes specifically hexokinase and phosphofructokinase cellular metabolic consumption continues at an attenuated rate as long as erythrocytes remain in direct contact with the overlying serum phase [14, 28]. Over prolonged post-centrifugation holding times, this persistent *in vitro* glycolytic flux steadily depletes serum glucose reserves.

The pronounced analytical bias observed in plain non-gel tubes after 48 hours exceeds the allowable clinical tolerance defined by the CLIA TEa criterion [23]. In clinical practice, an error of this magnitude introduces a critical pre-analytical hazard, predisposing patients to artifactual pseudohypoglycemia or masking clinically relevant hyperglycemia during routine diabetes mellitus monitoring [29, 30]. Such analytical discrepancies directly conflict with clinical consensus guidelines and can precipitate erroneous therapeutic adjustments or delayed medical interventions [29,30].

CONCLUSION

Serum Separator Tubes (SST) effectively preserve the stability of post-centrifugation serum glucose concentrations during delayed refrigerated storage at 2–8 °C for up to 48 hours without requiring manual aliquoting into secondary containers, maintaining an analytically negligible deviation (1.48%) that fulfills the Clinical Laboratory Improvement Amendments (CLIA) Total Allowable Error criterion of ±10%. Conversely, retaining unseparated serum directly in contact with the cellular clot in plain non-gel tubes leads to progressive, marked glycolytic depletion that reaches an 18.84% reduction at 48 hours, rendering such delayed specimens diagnostically unacceptable and prone to inducing false-negative or artifactual pseudohypoglycemic interpretations. A notable limitation of this study is its exclusive inclusion of normoglycemic healthy adult cohorts under tightly regulated laboratory refrigerator conditions, without evaluating severe dysglycemic states (e.g., severe hyperglycemia or hypoglycemia) or fluctuating transport temperatures encountered during sample logistics. Therefore, clinical and referral laboratories facing unavoidable processing backlogs or transport delays are strongly recommended to utilize gel-barrier primary tubes to safeguard diagnostic accuracy, while future studies should evaluate stability dynamics across diverse pathological blood glucose spectra and varied field storage environments.

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