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INNOVATIVE CLOSED TUBE PROTOCOL REVEALS A SUPER CRITICAL EARLY PREANALYTICAL PHASE OF WHOLE BLOOD GLUCOSE STABILITY IN ROUTINE MATRICES

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KEYWORDS

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Letter to the Editor

To the Editor,

Pre-analytical handling remains the dominant source of inaccuracy in glucose testing, particularly in decentralized or high-throughput pathways where draw-to-analysis delays and early manipulations are variable and rarely documented [1]. Most published data are derived from serum or plasma obtained after early separation and do not directly inform the in-tube behaviour of glucose in unopened whole blood during the first minutes after venipuncture a period of maximal metabolic activity and currently unsampled in most stability studies [2]. Existing evidence therefore quantifies only the long-tail drift occurring (tens of minutes to hours), whereas the short-latency kinetics immediately after collection remain largely uncharacterized [3]. In this context, matrix such as citrate buffer could improve stability of glucose by acidification leading to early inhibition of glycolysis acting on hexokinase optimal condition [4].

To address this unresolved interval, we designed a closed-tube, non-centrifuged, continuously mixed whole-blood protocol with repeated bedside measurements to eliminate transport delays, recapping artifacts and sedimentation-related phase separation [2]. 30 volunteers provided 4 different types of tubes of blood (VACUETTE® CAT serum fast separator 3.5 mL (REF454593), VACUETTE® LH heparin tubes 4 mL (REF485222), VACUETTE® FX Sodium Fluoride/Potassium Oxalate 4 mL (REF 454297) and VACUETTE® FC Mix tubes 3 mL (REF454513) (Greiner Bio-One, Kremsmunster, Austria).) which were collected by venipuncture, using VACUETTE® Evoprotect safety blood collection (Ref 450127) (Greiner Bio-One, Kremsmunster, Austria). The study was approved by the Hospitalo-Facultaire Ethics Committee of the University of Liège (reference: 707, September 23,2014), in the context of a general authorization to collect samples from healthy volunteers for technical studies, and was conducted in 2025 in accordance with the Declaration of Helsinki. This study was conducted over 4 separate days. Tubes were continuously gently mixed horizontally at room temperature on a tube roller device SCI-T6-S (Scilogex, USA) for all duration of the experiment to avoid natural sedimentation. To maintain anaerobic conditions, blood drops were collected every 20 min without opening the tubes, using the Haemo-Diff® device (REF 141217, SARSTEDT, Germany) Glucose was measured at baseline (T0) and every 20 min up to 120 min using a single point-of-care system (Nova StatStrip Xpress 2 glucometer point of care device, REF56506, Nova Biomedical®, Germany) [5]. Results were expressed as relative deviation from baseline and compared with Clinical Laboratory Improvement Amendments (CLIA) allowable total error.

Slopes were computed for the early (0-20 min) and later (20-120 min) segments to characterize time-structured kinetics. The percentage deviation from baseline ($PD\%$) was calculated for each measurement as: $PD\% = 100 * ((T“x” - T0))/T0$. Where Tx correspond to the test value at time x and $T0$ the baseline measurement in accordance with the recommendations for stability studies proposed by European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Working Group on Preanalytical Phase [6]. For repeated measures, comparisons were performed using the Friedman test, followed by Wilcoxon signed-rank post-hoc tests against $T0$. In addition, Wilcoxon signed-rank tests were applied to determine the earliest time point at which results exceeded the

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acceptability limits defined by the CLIA. These U.S. federal quality standards specify analyte-specific allowable total error, which was used here as the clinical acceptability threshold. This closed-tube, high-frequency design isolates intrinsic wholeblood kinetics without handling artifacts and allows direct resolution of the immediate post-draw period, a region functionally absent from most prior work.

Across the four matrices, glucose exhibited a timedependent decline except in the citrate-fluoride-EDTA (CFE) condition (Figure 1). In Fast Serum, lithium-heparin and fluoride-oxalate tubes, values were already lower than baseline at 20 min and exceeded the CLIA allowable total error (8 %) between 40 and 60 min. In contrast, CFE showed no significant deviation from baseline throughout the 120 min interval and never breached clinical acceptability. When restricting analysis to the initial 0-20 min interval, a super-critical early phase of glucose loss was resolved in all non-acidified matrices, whereas CFE preserved baseline values (Table 1). Early slopes were significantly negative in Fast Serum, lithium-heparin and fluoride-oxalate, but not in CFE, and pairwise contrasts confirmed that the separation in this window is essentially CFE vs. all other matrices. Beyond 20 min, slopes attenuated across all matrices and between-matrix contrasts no longer reached significance, indicating that the clinically relevant divergence originates within the immediate post-draw segment rather than during the later period.

This temporal structure explains the observed CLIA-limit crossings in non-acidified matrices and the persistent acceptability of CFE: in standard matrices most of the diagnostic error accumulates before the time points typically captured in conventional stability designs. Most published stability studies do not sample the first minutes after venipuncture and therefore cannot resolve the initial trajectory of whole-blood glucose before centrifugation [3]. The present design closed tubes, continuous mixing, repeated *in situ* measurements and absence of handling or transfer isolates intrinsic biochemical kinetics and demonstrates that the divergence between matrices occurs predominantly in a super-critical early phase, which is not captured by standard protocols. Only one historical study, published more than three decades ago, investigated glucose decay directly in whole blood [7]. While pioneering for its time, this work had several important limitations. First, the measurements required opening the tubes at each time point and transferring aliquots to a laboratory analyzer, introducing handling delays and oxygen exposure that could confound the true kinetics. Second, the sampling schedule started at 15 min, missing the very early phase immediately after venipuncture. Third, the study focused exclusively on fluoride and heparin tubes, without evaluating more recent matrices such as citrate-fluoride-EDTA. Our study overcomes these limitations by using a closed-tube, continuously mixed, spin-free protocol with bedside point- of-care devices, enabling a genuine $T0$, dense sampling from the first minutes, andparallel evaluation of glucose across four common matrices. This design allowed us to expose a previously underappreciated early critical phase of glucose instability and to demonstrate that CFE can simultaneously secure glucose within clinically acceptable limits.

This finding extends current evidence in two ways. First, it shows that clinically relevant error accumulates before the time anchors commonly addressed in existing guidance (e.-g. ≤30 min to centrifugation or delayed plasma sampling). Second, it confirms that acidified citrate-fluoride-EDTA

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prevents this early loss under whole-blood conditions [8], which explains its persistent compliance with CLIA criteria across the full observation window. These observations are methodologically compatible with prior reports of improved stability in citrate-buffered systems, but refine the timing and mechanism by showing that the protective effect principally applies to the unresolved early interval rather than the later phase typically measured.

However, several limitations warrant consideration. The study was confined to healthy adults under controlled conditions, used a single point-of-care platform without reference-method comparison and outside the specifications provided by the manufacturer, and did not test populations with leukocytosis and according to erythrocytes count [9], extreme glucose values or altered hematocrit, all of could merit specific evaluation.

By resolving early in-tube kinetics in unopened, continuously mixed whole blood, this study identifies a super-critical early phase of glucose loss in non-acidified matrices and demonstrates that citrate-fluoride-EDTA prevents this early divergence within clinically acceptable limits. These findings refine the temporal understanding of pre-analytical instability and support future confirmation in broader clinical settings. Beyond glucose, many labile metabolites may also exhibit a super-critical early kinetic phase in whole blood; systematically mapping this interval and choosing matrices that arrest metabolism, including acidified systems such as CFE, could provide more faithful baselines and safer clinical interpretation across a wider test menu. This study offers a practical template for modernizing pre-analytical policy toward matrix-based control of early kinetic risk in contemporary, decentralized pathways.

***Figure 1**. Time-course variation of whole-blood glucose across matrices. Relative change from baseline (T0) is expressed as % variation (mean ± SD). The red dashed line indicates the CLIA allowable total error for glucose (8 %). FS and LiH declined progressively, crossing CLIA acceptability by 60 min. FOx displayed a steep early decline and exceeded CLIA limits by 40 min. CFE remained stable without significant time effect and never breached CLIA limits over 120 min.*

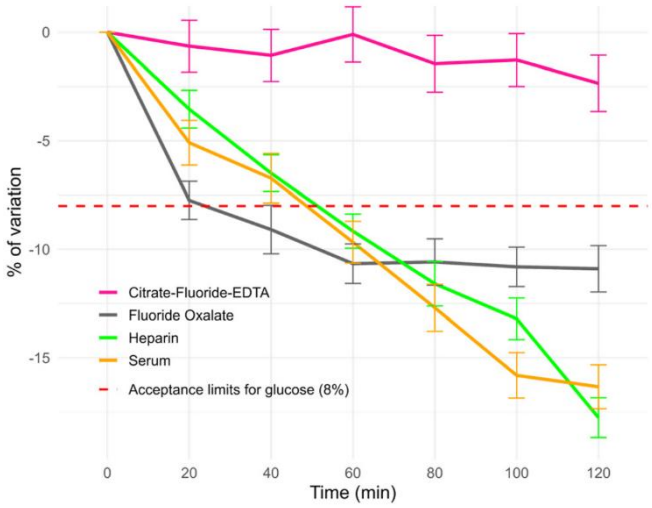

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***Table 1**. Short-term slopes of whole-blood glucose by matrix, with comparisons between matrices.*

Interval, min	n	FS slope, mmolL^{-1} h^{-1}	p-Value (compared to 0 slope)	LiH slope, mmolL^{-1} h^{-1}	p-Value (compared to 0 slope)	FOx slope, mmolL^{-1} h^{-1}	p-Value (compared to 0 slope)	CFE slope, mmolL^{-1} h^{-1}	p-Value (compared to 0 slope)	p (FOx vs. CFE)	p (FOx vs. LiH)	p (FOx vs. FS)	p (LiH vs. FS)	p (LiH vs. CFE)	p (FS vs. CFE)
0–20	30	−0.74 ± 1.9	0.022	−0.89 ± 1.9	0.013	−1.12 ± 1.1	0.00002	0.15 ± 1.7	0.61	0.0012	0.51	0.71	0.92	0.02	0.02
40–60	30	−0.07 ± 1.8	0.647	0.3 ± 1.9	0.59	−0.36 ± 2.5	0.488	−0.26 ± 2.0	0.34	0.51	0.07	0.24	0.45	0.37	0.77
60–120	30	−1.07 ± 3.3	0.052	0.1 ± 3.04	0.44	0.08 ± 3.8	0.85	−0.07 ± 3.8	0.99	0.93	0.43	0.06	0.11	0.94	0.14

FS, fast serum; LiH, lithium-heparin; FOx, fluoride–oxalate; CFE, citrate–fluoride–EDTA. Unit of slope: use mmol·L^{-1}·h^{-1} (based on relative change) but keep it consistent across the table. Negative values indicate decline. Mean ± SD.

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