Research Article

Comparison of the glucose stability effect between Citrate–sodium fluoride–EDTA, Heparin, and Sodium fluoride containing tubes: a prospective experimental study in the Biochemistry Laboratory at the University Clinic of Kinshasa, DR Congo

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Keywords

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Abstract

Tubes containing sodium fluoride (NaF) are no longer recommended for measuring blood glucose levels. This prospective experimental study was conducted at the Biochemistry Laboratory of the University Clinics of Kinshasa,in the Democratic Republic of the Congo, and included seventy healthy volunteer blood donors. Glucose stability was assessed in tubes containing NaF,lithium heparinised tubes, and a tube containing a mixture of citrate,NaF and EDTA.The tubes containing citrate and NaF were stored at room temperature,whilst the corresponding heparinised tubes were placed on ice. Glucose measurements were taken at various time points (H0, H2, H24, H48) on centrifuged plasma kept in contact with blood cells.The citrate showed the highest and most stable glucose values over time,with virtually no loss of glucose.Lower glucose values were observed at H0 and H2 with the NaF ($p < 0.05$).Transporting the heparin tube on ice combined with centrifugation at +4°C resulted in higher blood glucose values than the NaF at time points H0 and H2 ($p < 0.05$),although lower than the citrate($p < 0.05$);however, these values could not be maintained beyond 24 hours.Positive biases exceeding the desirable bias (1.8%) were observed between citrate and NaF tubes,as well as between heparinised and NaF tubes;this suggests a marked discrepancy and, consequently,a clinically significant difference between the various methods.For accurate blood glucose measurement,the citrate-NaF-EDTA tube is preferable in order to minimise glucose loss; however,a review of the diagnostic thresholds for diabetes mellitus must be carried out before its use.Cooling the sample is an effective alternative to compensate for the lack of citrate.

Introduction

Blood glucose is a key parameter for the diagnosis of diabetes mellitus, particularly in resource-limited settings. Accurate measurement of blood glucose is essential for appropriate patient management [1]. However, during the analytical process of glucose determination, several pre-analytical factors [2] may influence glucose concentration in the sample, including factors related to the patient's physical condition, those associated with sample handling and storage, and an intrinsic factor, namely glycolysis.

Glycolysis is a physiological process that occurs in the presence of blood cells and consists of glucose degradation through a series of enzyme-catalyzed chemical reactions, resulting in the production of energy and metabolites, including pyruvate, which is essential for the functioning of living organisms [3]. This process continues in vitro after blood collection and may be enhanced by prolonged contact time between blood cells and plasma [2,4,5], environmental temperature and hydrogen potential surrounding the blood cells [6], and sample cellularity [7,8].

When glycolysis is not controlled in blood samples, a decrease in plasma glucose concentration of 5–7% per hour may occur, corresponding to approximately 0.6 mmol/L or 0.1 g/L [9]. This may lead to misclassification of diabetic patients and, consequently, inappropriate clinical management.

To mitigate the impact of glycolysis during blood glucose analysis, various physical and chemical approaches have been recommended, including immediate separation of plasma from blood cells by centrifugation, the use of antiglycolytic additives, or immediate placement of collected blood samples on ice followed by centrifugation within 15 to 30 minutes [2,10,11]. The latter procedure has proven to be less practical in routine practice due to the required materials and equipment [12]. Among glycolysis inhibitors, sodium fluoride coated in blood collection tubes remains one of the most widely used in medical laboratories. Sodium fluoride (NaF) inhibits enolase, an enzyme involved in the final step of glycolysis [4]. However, several studies have demonstrated that the antiglycolytic effect of NaF becomes effective only two to four hours after phlebotomy [4,13,15], resulting in glucose loss in collected blood samples estimated at approximately 0.1 g/L per hour [16].

Consequently, recent recommendations from the American Diabetes Association (ADA) and the American Association for Clinical Chemistry (AACC), published in 2023 and updated in 2024, advocate the use of citrate for glycolysis inhibition as a replacement for NaF [1]. Citrate, through blood acidification, immediately halts in vitro glycolysis by inhibiting early glycolytic enzymes, including hexokinase and phosphofructokinase [14]. The antiglycolytic effect of citrate is maintained for approximately ten hours at room temperature [17]. However, the use of citrate in liquid form leads to dilution of the blood sample. The granular form of citrate, as contained in Vacuette® FC Mix tubes, overcomes this limitation. In addition to citrate, these tubes contain sodium fluoride associated with potassium oxalate (NaF/KOX) and ethylenediaminetetraacetic acid (EDTA) as an anticoagulant; the mixture of these additives is presented in the form of fine granules. According to several studies [11,15,18], in addition to ensuring complete inhibition of glycolysis through the combined inhibitory action of citrate and NaF, Vacuette® FC Mix tubes prevent the dilution effect [11].

Vacuette® FC Mix tubes are not available in the Democratic Republic of the Congo. To date, NaF/KOX-containing blood collection tubes remain the standard practice for blood glucose determination in the Biochemistry Laboratory of the University Clinics of Kinshasa.

This study aims to compare glucose stability among citrate–sodium fluoride–EDTA, lithium heparin, and sodium fluoride-coated blood collection tubes in a laboratory setting.

Materials and Methods

Study population

This prospective experimental study was conducted in accordance with the principles of the Declaration of Helsinki, revised in 2024, and was approved by the Ethical Committee of the School of Public Health at the University of Kinshasa (ESP/CE/42/2025).

A cohort of seventy participants, including both men and women, was consecutively recruited among voluntary blood donors attending a blood donation campaign organized in March 2025 by the Blood Bank of the University Clinics of Kinshasa, Democratic Republic of the Congo. The blood donors included in the study were those who were considered apparently healthy and non-diabetic according to routine pre-donation screening criteria [19] and provided informed consent.

Sample collection

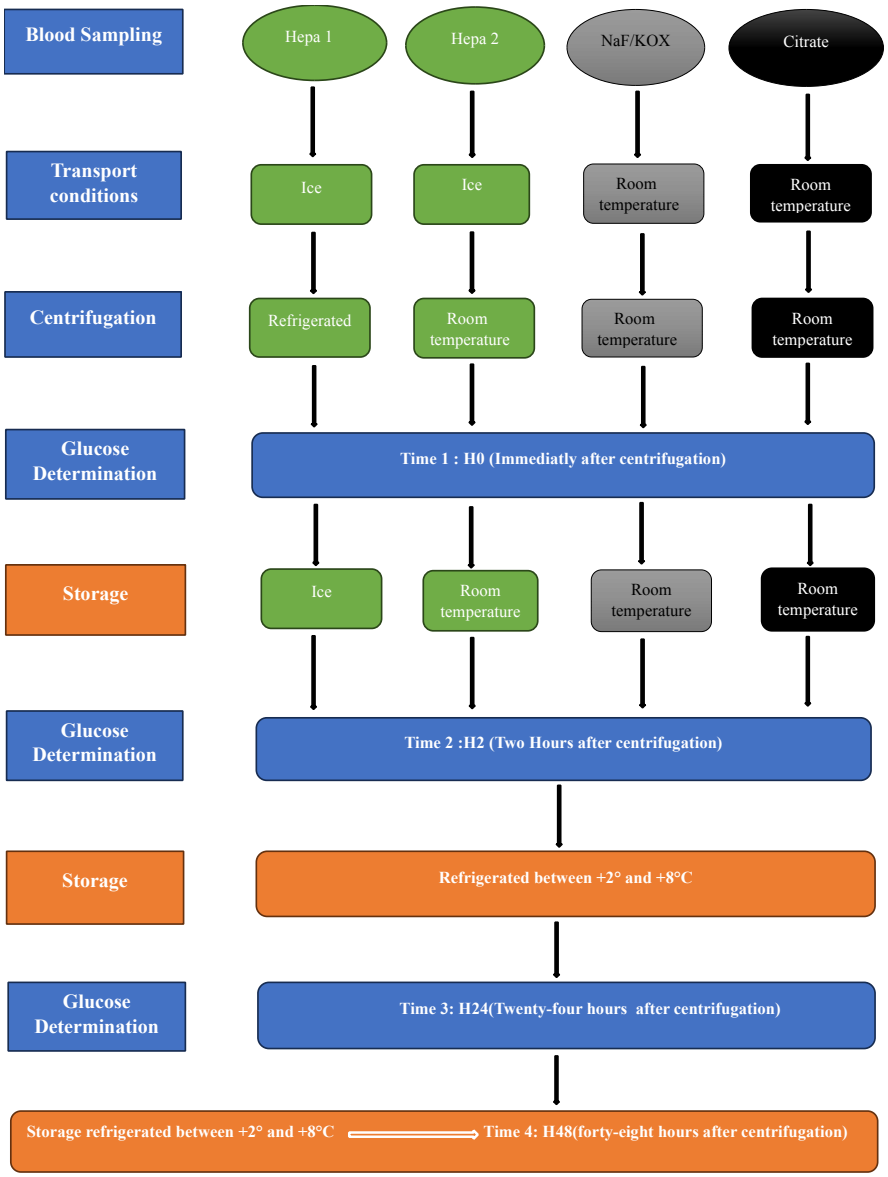
For each participant, after blood donation, peripheral venous blood was collected into four blood collection tubes: two lithium heparin–coated tubes (VACUUBLOOD®, provided by AMT Pharma, DR Congo), hereafter referred to as Hepa 1 and Hepa 2 tubes; one sodium fluoride and potassium oxalate–coated tube (VACUUBLOOD®, provided by AMT Pharma, DR Congo), referred to as the NaF/KOX tube; and one tube coated with a mixture of ethylenediaminetetraacetic acid (EDTA), sodium fluoride and potassium oxalate (NaF/KOX), and citric acid–sodium citrate (VACUETTE® FC Mix, provided by Greiner Bio-One, France), referred to as the citrate tube.

Sample transportation, pretreatment, and glucose analysis

From the sampling, the Hepa 1 tube was transported on ice to the Genetics Laboratory of the Faculty of Medicine at the University of Kinshasa within a maximum of 10 minutes for centrifugation at +4°C (MEGAFUGE 16R refrigerated centrifuge, Thermo Scientific, China). It was then sent to the Biochemistry Laboratory of the University Clinics of

Kinshasa within a maximum of 30 minutes for various blood glucose tests (15 minutes for centrifugation and 15 minutes for transportation).
Furthermore, the Hepa 2 tubes were transported on ice, while the Citrate and NaF tubes were transported at room temperature. These last three tubes were sent directly to the Biochemistry Laboratory of the University Clinics of Kinshasa for centrifugation at room temperature (SLOTEQ centrifuge, serial number W1920001906596, Finland) and for various blood glucose measurements within a maximum of 30 minutes. On centrifuged plasmas kept in contact with blood cells, Glucose measurements were performed using the MINDRAY BS240 analyzer (MINDRAY BS240, YX91002321, Shenzhen, China) with glucose oxidase–peroxidase reagents supplied by Mindray. Prior to glucose measurement, calibration and internal quality control procedures were performed using the Mindray Multi Sera Calibrator (lot 150124006) and Mindray Clinchem Multi Control level 1 (lot 0593224013) and level 2 (lot 059424010).Glucose concentrations were determined at four different time points: time zero (H0), immediately after centrifugation; time one (H2), 2 hours after centrifugation; time two (H24), 24 hours after centrifugation; and time three (H48), 48 hours after centrifugation.After H0 glucose measurement, the Hepa 1 tube was maintained in contact with ice, whereas Hepa 2, NaF/KOX, and citrate tubes were stored at room temperature. After H2 measurement, all primary tubes were stored at temperatures between +2 and +8 °C pending H24 and H48 glucose measurements.

Figure 1: The flowchart describe the pre analytical and the analytical process of glucose determination.



**Centrifugation: 2200 revolutions for 15 minutes (technical specifications of sampling tubes and compilation of data from similar studies).*

Data analysis

Statistical analyses were performed using Stata Statistical Software: Release 18 (StataCorp, 2023), and graphical representations were generated using R software, version 4.4.2 (2024). Comparisons between paired tubes were conducted using the Wilcoxon signed-rank test. Agreement between measurements was assessed using Bland–Altman plots, constructed for each tube pair comparison at each measurement time point. A Passing-Bablok regression was also performed to validate the results obtained using the Bland-Altmann plot. The effects of tube type and analysis time on blood glucose concentrations were evaluated using a generalized linear mixed model (GLMM). All analyses were interpreted using a 95% confidence interval, with the level of statistical significance set at 0.05.

Results

As blood glucose values were not normally distributed, they were described using the median and interquartile range (IQR). Table 1 presents the median blood glucose values obtained for the different tube types at the various measurement time points (H0, H2, H24, and H48).

Table 1: Distribution of blood glucose levels (Median and IQR) in mmol/L across different types of tube and at four time points.

Tubes	Median glucose (mmol/L)			
	H0 (IQR)	H2 (IQR)	H24 (IQR)	H48 (IQR)
Hepa1	5.47(1.13)	5.46(1.11)	5.20(1.28)	4.64(1.31)
Hepa2	5.38(1.17)	5.35(1.22)	4.61(1.27)	4.13(1.41)
NaF/KOX	5.32(1.21)	5.29(1.22)	5.26(1.27)	5.35(1.30)
Citrate	5.67(1.24)	5.60(1.18)	5.64(1.23)	5.63(1.30)

Table 2 and Table 3 illustrates respectively the statistical significance ($p < 0.05$) of the differences in blood glucose concentrations observed in citrate, Hepa 1, and Hepa 2 tubes compared with the NaF/KOX tube and the differences in blood glucose concentrations observed in NaF, Hepa 1, and Hepa 2 tubes compared with the Citrate tube.

Table 2: Median blood glucose(mmol/L) levels in different tube compared with the NaF tube (Wilcoxon test) at each time point.

Tubes	Median (mmol/L)	IQR	p_value
Hepa1			
H0	5.47	1.13	<0.001
H2	5.46	1.11	<0.001
H24	5.2	1.28	<0.001
H48	4.64	1.31	<0.001
Hepa2			
H0	5.38	1.17	<0.001
H2	5.35	1.22	0.049
H24	4.61	1.27	<0.001
H48	4.13	1.41	<0.001
Citrate			
H0	5.67	1.24	<0.001
H2	5.6	1.18	<0.001
H24	5.64	1.23	<0.001
H48	5.63	1.3	<0.001

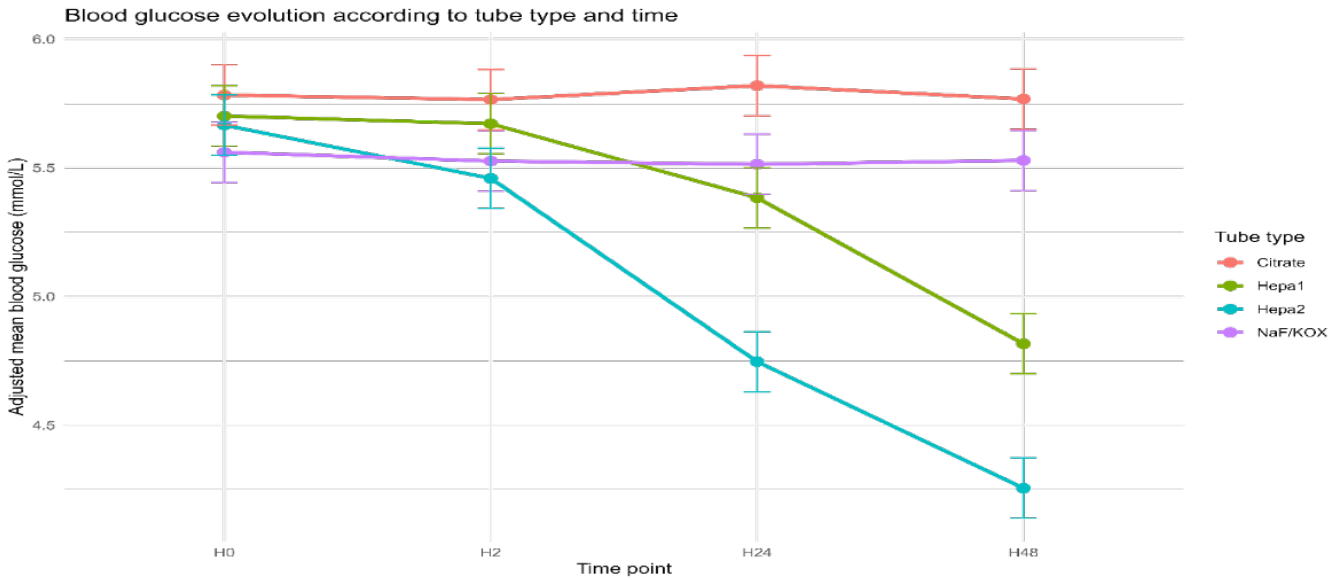
Table 3: Median blood glucose(mmol/L) levels in different tube compared with the Citrate tube (Wilcoxon test) at each time point.

Tubes	Median (mmol/L)	IQR	p_value
Hepa1			
H0	5.47	1.13	0.021
H2	5.46	1.11	0.001
H24	5.2	1.28	<0.001
H48	4.64	1.31	<0.001
Hepa2			
H0	5.38	1.17	<0.001
H2	5.35	1.22	<0.001
H24	4.62	1.27	<0.001
H48	4.14	1.41	<0.001
NaF			
H0	5.32	1.21	<0.001
H2	5.3	1.22	<0.001
H24	5.6	1.27	<0.001
H48	5.35	1.3	<0.001

The highest blood glucose concentrations were observed in the citrate tube at all measurement time points with a markedly significant difference(P<0.05) compared with the other tubes.
In the early measurement periods (H0 and H2), the Hepa 1 tube, for which the cold chain was maintained from collection to the H0 measurement, yielded blood glucose values lower than those of the citrate tube but higher than those of the NaF tube, a significant difference ($p < 0.05$) (Table 3).

The NaF tube gave low blood glucose values in the first two hours, with a significant difference compared to the citrate tube (P<0.05).
The Hepa1 and Hepa2 tubes showed decreasing blood glucose values beyond the H2 measurement, with a significant difference compared to the citrate tube (P<0.05).
Glucose stability over time across the different tube types is illustrated in Figure 2.

Figure 2: Adjusted mean blood glucose according to tube type and time of analysis, estimated using a generalized linear mixed model (GLMM).



Glucose stability was observed from H0 to H48 in the citrate and NaF tubes. In contrast, the Hepa 1 and Hepa 2 tubes showed a progressive and significant decrease in blood glucose ($p < 0.001$).
Figure 3 presents the kinetics of glucose degradation according to the analysis time and tube type, the initial glucose value measured at the first time point (H0) for each tube type.
Quantification of glucose losses for each tube at the different measurement time points, using H0 as the baseline, is presented in Table 4.

Figure 3: Loss of blood glucose over time for each tube type.

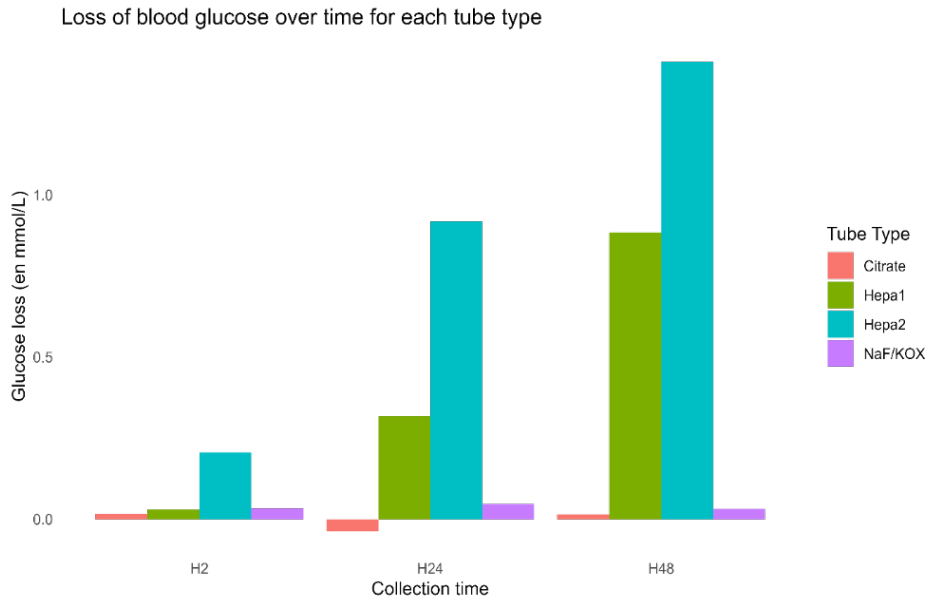


Table 4: Glucose loss comparison by tube and time. Estimated difference (%) compared to H0 with 95% CI.

	Estimate (%) [CI]	p_value	Signif
Hepa1			
H2	0.41 [-1.10; 1.92]	0.595	ns
H24	5.45 [3.93; 6.96]	<0.001	***
H48	15.62 [14.11; 17.13]	<0.001	***
Hepa2			
H2	3.64 [2.30; 4.99]	<0.001	***
H24	16.47 [15.12; 17.82]	<0.001	***
H48	25.26 [23.91; 26.61]	<0.001	***
NaF/Kox			
H2	0.62 [-0.18; 1.42]	0.1283	ns
H24	0.70 [-0.10; 1.49]	0.0872	ns
H48	0.52 [-0.28; 1.31]	0.2049	ns
Citrate			
H2	0.27 [-0.70; 1.24]	0.582	ns
H24	-0.75 [-1.71; 0.22]	0.132	ns
H48	0.22 [-0.75; 1.18]	0.662	ns

**** :P<0.05(Significative difference) ; ns : non significative difference.*

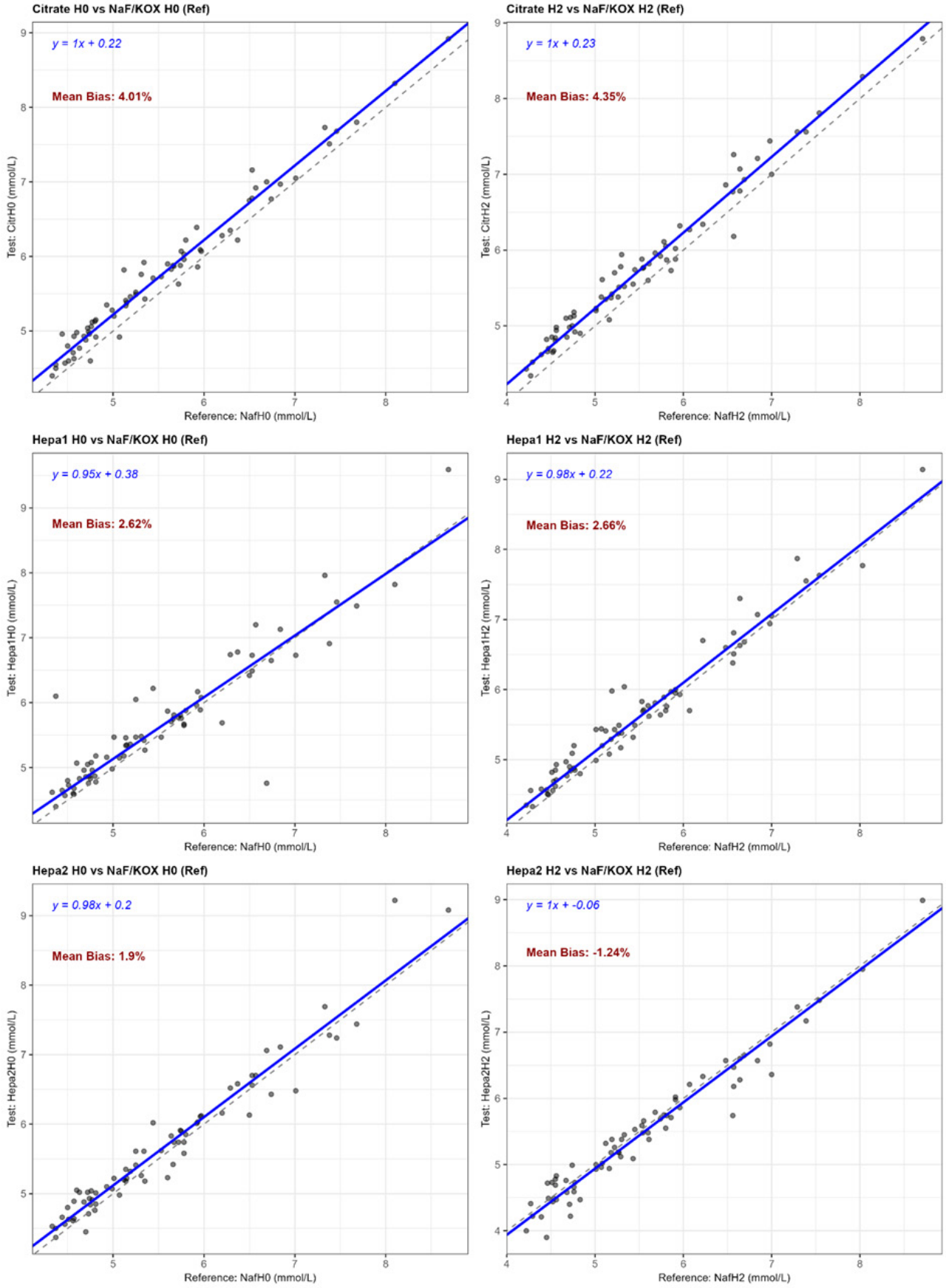
Over time, glucose losses were significant in the Hepa 1 and Hepa 2 tubes ($p < 0.05$); For the Hepa 1 tube, this was observed beyond H2, and for the Hepa 2 tube from the very start of the assay. In contrast, good glucose stability was observed with the Citrate and NaF tubes (losses ≤1%) and we noted a P value >0.05, non-significative.

Table 5: Agreement between alternative blood collection tubes and the NaF : A Bland-Altman analysis; Desirable bias at 1.8% [20].

Comparaison	Time	Bias Percent (%)	SD_Percent (%)	LoA_lower_percent	LoA_upper_percent
Citrate	H0	4.01	3.06	-1.99	10
Citrate	H2	4.35	3.02	-1.57	10.28
Hepa1	H0	2.62	7.01	-11.13	16.36
Hepa1	H2	2.03	3.75	-5.33	9.38
Hepa2	H0	1.9	3.85	-5.64	9.45
Hepa2	H2	-1.88	3.98	-9.67	5.92

Table 5 shows the degree of agreement between the citrate tubes and the NaF tubes, the Hepa 1 tubes and the NaF tubes, and the Hepa 2 tubes and the NaF tubes at the H0 and H2 measurement times. The comparison biases for each pair of tubes were compared with the target bias provided by the database of chemical performance specifications derived from the consensus of the European Federation of Clinical Chemistry and Laboratory Medicine and Westgard (target bias 1.8% for glucose) [20]. We note, in the first few hours, a discrepancy between citrate and NaF, as well as between heparinised tubes and NaF. The same conclusions are drawn using Passing-Bablok regression, as illustrated in the following figure. The statistical differences observed between the citrate-NaF and heparin-1-NaF tubes are likely, if not certainly, associated with actual clinical differences.

Figure 4: Passing-Bablok regression analysis and mean percentage bias of plasma glucose levels measured in different collection tubes compared to the NaF tube reference.



Discussion

In this study, the NaF tube was compared with the granular mixture of citrate, sodium fluoride, and ethylenediaminetetraacetic acid contained in the Vacuette® FC Mix tube (citrate tube), as well as with lithium heparin tubes stored on ice (Hepa 1 and Hepa2). The citrate tube yielded the highest blood glucose values compared with the other tube types, with near-zero and non-significant glucose losses over time. This demonstrates that blood acidification enables early, complete, and sustained inhibition of glycolysis for up to 48 hours. Indeed, each reagent contained in the citrate tube contributes to the prolonged antiglycolytic effect.

Furthermore, the citrate-containing tubes used in our study were in granular form. This is advantageous, as it avoids any dilution effects on glucose concentration compared with tubes containing citrate in liquid form [21,22]. This result is in line with previously published data Fokkert et al. [23], Uchida K et al [24], Gambino et al [25], Rebecca Carey et al. [26], Graziella Bonetti et al [27], Patil S et al [28], Gupta S et al [29] , Ridefelt P et al[30].

Higher glucose measures observed with the use of citrate coated tubes, raise some diabète mellitus diagnostic issues, depending on the population involved. The study by del Pino et al [31] reported a significant raise of positive gestational diabète mellitus after oral glucose tolerance test; but the difference was not significant in general patients . Riefelt P et al used data of patients from clinical routine health care and observed increased values of glycemia above the diabete decision limit after implementation of citrate coated tubes [30]. The overestimated plasma glucose level observed with citrate tube is not due to glucose increase, but only to the related more accurate glucose preservation in the blood sample. Consequently, usage of citrate tubes can increase the prevalence of impaired fasting glucose as well as the diagnosis of diabetes mellitus since glucose levels are closer to the diagnostic thresholds. In contrast, the study by Fisher M et al. [32] did not report on glucose overestimation with the citrate tube. The difference in methodology could explain the result size of the studied population and also samples storage at room temperature.

The same result was reported by Van der Hagen et al. [11], who included pregnant women referred for gestational diabetes screening. However, they observed that the blood glucose values obtained using the citrate tube correlated perfectly with those obtained using the NaF/EDTA tube, with the Bland-Altman plot showing a non-significant bias of 0.06 mmol/L before and after glucose loading during an oral glucose tolerance test in a cohort of pregnant women. In our study, we observed biases exceeding the acceptable limit when comparing the different tubes against NaF [20]. The assessment of citrate-NaF agreement revealed a bias of approximately 4% (Table 5), this bias having been analysed only for measurements taken at time points H0 and H2. Tiago Tizziani et al. in their study also observed that the two methods(Citrate and NaF/EDTA) Practically presented no correlation ($r = 0.15$)[33].In view of these results, citrate and NaF tubes do not agree, just as with the comparison of heparinised tubes and NaF. Whilst citrate tubes do provide optimal blood glucose values for better management of diabetes mellitus [1], there is a need to initiate discussions on adjusting the diagnostic thresholds for diabetes mellitus (DM) with these new (citrate) tubes.

Although the NaF tube showed slightly lower median blood glucose levels than the citrate tube, with a statistically significant difference ($P<0.001$), it demonstrated good glucose stability over time, matching the citrate tube, which showed optimal stability.

Given the delayed antiglycolytic effect of NaF, one would expect glucose losses to be significant over time, particularly at H2. However, Gambino et al. [25] reported significant glucose losses of 4.6% at H2 with the NaF tube. This discrepancy with our results may be explained by methodological differences. In Gambino et al., aliquots were stored at 37 °C, with the elevated temperature serving as a factor that exacerbates glycolysis.

During the first few hours, the Hepa 1 tube yielded higher median blood glucose values than the NaF tube ($P < 0.05$) and slightly lower values than the citrate tube ($P < 0.05$), with non-significant glucose losses ($P = 0.595 > 0.05$ at H2). This result may be explained by the fact that the Hepa 1 sample was transported in an ice slurry, centrifuged at +4 °C and kept in contact with ice until the H2 measurement, with the cold chain exerting an early inhibitory effect on the collected sample. However, this procedure proved less practical in routine practice due to the materials and equipment required, which are not always available in our setting [12]. Consequently, another laboratory had to be commissioned to carry out the refrigerated centrifugation of these samples from our cohort.

Recommendations advise separating plasma from cells within 15 to 30 minutes of blood collection [2]; however, strict adherence to this protocol is difficult to implement in routine practice. We encountered the same limitation in our study: although the plasma was kept in contact with the blood cells without decanting the sample, it took us a long time before the first measurement due to the distance to the centrifugation site. This could explain why the chilled Hepa1 tube, which was transported on crushed ice to the laboratory, proved less effective than the citrate tube.

Rebecca Carey et al. [26] reported similar glucose values between lithium heparin and sodium fluoride tubes, likely because both tubes were placed on ice immediately after collection, in contrast to our protocol.

This study is among the first conducted in our setting, as citrate mixture–coated tubes are recommended for blood glucose determination.

A limitation of the study is that the study population comprised only healthy individuals. Further studies of citrate tubes in our context, within a population suspected of having diabetes

mellitus or diagnosed with diabetes, would be necessary in order to better understand the clinical differences between citrate tubes and NaF tubes and to draw definitive conclusions. Cases of diabetes mellitus would likely increase with citrate tubes, given their anti-glycolytic efficacy in vitro.

Conclusion

In conclusion, among NaF and heparin containing tubes, the citrate-NaF-EDTA coated tubes appear to have the most stable glycolyse inhibitor effect over time, in our laboratory-based study. If citrate tubes are not available, using an ice slurry to transport samples, or better still, refrigerated centrifugation, would be a good alternative. The current diagnostic thresholds based on blood samples collected in tubes containing only NaF are probably too low.A reassessment of the decisive diagnostic thresholds will have to be carried out with the citrate tube and, why not, also with the tube preserved in ice.

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Data Availability

All data are available from the corresponding author upon reasonable request.

Declaration of conflict of Interest

Authors do not have any conflict of interest.

Ethical Approval

Ethical approval was obtained by the research panel (ethics number ESP/CE/42/2025).

Authors contributions

-Ongona Sheko contributed to conceptualization, data curation, formal analysis, investigation, validation, methodology, visualization, writing – original draft.
-Ngole Mamy contributed to conceptualization, data curation, formal analysis, investigation, validation, methodology, visualization, writing – original draft.
- Pambu Dahlia and Tshibuela Beya Dophie contributed to review and editing.
-Mukadi Patrick and Beya Michael participated in the statistical analysis.

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Supplemental material

*Lithium heparin tubes: VACUUBLOOD®, provided by AMT Pharma, DR Congo, *Fluoride and potassium oxalate tube: VACUUBLOOD®, provided by AMT Pharma, DR Congo. *Tube coated with a mixture of ethylenediaminetetraacetic acid (EDTA), sodium fluoride and potassium oxalate (NaF/KOX), and citric acid–sodium citrate: VACUETTE® FC Mix, provided by Greiner Bio-One, France-

* Centrifuges: -MEGAFUGE 16R refrigerated centrifuge, Thermo Scientific, China. -SLOTEQ centrifuge, serial number W1920001906596, Finland.
*The MINDRAY BS240 analyzer: MINDRAY BS240, YX91002321, Shenzhen, China with glucose oxidase–peroxidase reagents supplied by Mindray.

*Calibration and internal quality control: Mindray Multi Sera Calibrator (lot 150124006), from china and Mindray Clinchem Multi Control level 1 (lot 0593224013) and level 2 (lot 059424010) from China.