Practical Laboratory Medicine 39 (2024) e00388

Contents lists available at ScienceDirect

Practical Laboratory Medicine

journal homepage: www.elsevier.com/locate/plabm

Optimizing D-mannose and glyceraldehyde concentrations as glucose preservatives without clinically affecting biochemical test results

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ARTICLE INFO

Keywords:
Blood sample stability
Diabetes management
Glycolysis inhibition
Pre-analytical error
Preservative efficacy
Clinical criteria

ABSTRACT

Objectives: The objectives were to evaluate blood additives that combined lithium heparin (LH)-salt with glyceraldehyde (GLY) or D-mannose (MAN) for preserving glucose levels in plasma samples and to simultaneously determine the compatibility of these additives with 14 other biochemical tests.

Methods: Blood samples from 40 subjects, equally divided into healthy and diabetic groups, were collected using five different additives. The three most effective additives, LH/GLY, LH/MAN, and LH/GLY/MAN, were selected for ensuring the best preservation of glucose levels and compatibility with 14 biochemical tests. One-way analysis of variance was used to analyze the mean paired differences of glucose level and biochemical tests. Simultaneously, the clinical criteria from Johns Hopkins Hospital were used to guide the interpretation and set acceptable thresholds for measurements that exceeded the standards.

Results: The combination of 160 mmol/L GLY, 8.4 mmol/L MAN, and LH, maintained glucose levels at approximately 93.4–93.7 % for healthy subjects and 91.3–92.8% for subjects with diabetes mellitus over 8 h. The mean paired differences of glucose levels in preservation were statistically insignificant. The biases in 14 biochemical tests for LH/GLY/MAN and LH/MAN remained within the acceptable clinical criteria during the 8 h.

Conclusions: Combining 160 mmol/L GLY, 8.4 mmol/L MAN, and LH, proved more effective in maintaining glucose levels than individual additives or the conventional sodium fluoride preservative. It did not yield clinical discrepancies in the 14 biochemical tests during 8 h at room temperature.

1. Introduction

Glucose in lithium heparin (LH) and sodium fluoride (NaF)/potassium oxalate plasma is difficult to accurately preserve because of glycolysis if not immediately centrifuged, plasma aliquoted and analyzed [1]. Rapid plasma separation from cells is superior for

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https://doi.org/10.1016/j.plabm.2024.e00388
Received 23 February 2024; Accepted 9 March 2024
Available online 13 March 2024

preventing *in vitro* reductions in measured blood glucose concentration [2]. However, this is not feasible for clinical laboratory practice. NaF in combination with an anticoagulant has been recommended for blood specimen collection for plasma glucose in the diagnosis and management of diabetes mellitus (DM) [3–5]. NaF has been reported to be an ineffective glycolysis inhibitor, especially within the first 4 h of blood draw [6–8]. Table 1 summarizes blood additives and their impact on glucose and biochemical testing Insights from previous studies.

Various blood additives, including anticoagulants in combination with antiglycolytic agents, have been introduced for glucose measurements in clinical laboratories to increase the stability of glucose and minimize pre-analytic errors [9]. Glucose-specific tubes containing NaF/Potassium oxalate/EDTA, glyceraldehyde (GLY), and citric acid/citrate have been proven effective at minimizing glycolysis [10–14], D-mannose (MAN) alone or in combination with other anticoagulants, can adequately maintain glucose levels [15–17]. However, these studies have highlighted potential concerns regarding interference with biochemical tests. This study aimed to evaluate blood additives that combined LH salt with GLY or MAN for preserving glucose levels in plasma samples and to simultaneously determine the compatibility of these additives with 14 other biochemical tests.

2. Material and methods

2.1. Reagent preparation

2.1.1. Mannose solution

A 10 mL of distilled water (pH 7.35–7.45), containing 3 mg of MAN was prepared. Subsequently, 10 μL of this solution was added to a 1 mL blood sample [15].

2.2. Glyceraldehyde solution

A stock solution (250 mmol/L); 0.225 g of GLY was diluted to a working concentration and used within 1 h [10].

2.3. Combinations of heparin and antiglycolytic agents

Table 2 provides an overview of the five blood samples that contained heparin and glycolytic inhibitors and were used for glucose determination. In this study, the blood additives in tube number 5 were modified and further developed. The NaF/K_3EDTA tube and the LH tube were used as experimental controls.

Table 1
Blood additives and their impact on glucose and biochemical testing: Insights from previous studies.

Blood additives	Authors	Concentrations	Glucose stability	Advantage	Disadvantage
NaF [4]	Chan et al. (1989)	8–20 mg/dL (1% NaF+0.2 % Oxalate)	Completely inhibits glycolysis at the 4th h	Preserves glucose for 3 d.	- Hemolysis - Does not prevent loss of plasma glucose during the first 30–90 min after blood collection
MAN [15]	Nakashima et al. (1987)	Heparin +200–400 mg/dL of MAN (200 mg/dL was most effective)	4 h	No effect on electrolytes and enzymes	–
MAN [16]	Chan et al. (1992)	Combination 16.6 mmol/L of MAN (3 g/L) and NaF (6 g/L)	Decreasing range after 2 h is 0.08–0.44 mmol/L whereas NaF ranged 0.3–0.78 mmol/L	No hemolysis No interference in regular biochemical tests	- Positive interference by glucose oxidase method - Negative interference by Hexokinase method - Unsuitable for electrolyte (Na^+, K^+)
MAN [17]	Mithu et al. (2018)	3 mg/mL of MAN and heparin	After 3 h decreased <1%	No hemolysis	Interfered with potassium test
GLY [10]	Landt, M. (2003)	10 mmol/L	8 h	No effect on glucose oxidase and hexokinase method and electrolytes	- Fresh preparation required for glucose test - Increased creatinine (alkaline picrate method) - Decreased Alanine aspartate (AST) test
GLY [11]	Le Roux et al. (2014)	11 mmol/L of GLY + 119 mmol/L NaF + 21.7 mmol/L $K_2C_2O_4 \bullet H_2O$	48 h	No hemolysis and prevent glycolysis for 4 h	GLY alone does not inhibit glycolysis completely. - Fresh preparation required for glucose test
D-L GLY [13]	Ganapathy et al. (2016)	5 mmol/L of GLY	8 h	Very slightly decreased of glucose level	Creatinine by modify Jaffe's method and potassium test by Ion selective electrode method reported positive interference.

Table 2
Characteristic of anticoagulants and antiglycolytic agents employed in blood sample collection.

Additive container	Abbreviation	Additive details	Blood volume (mL) recommend by manufacturer
1	NaF/K_3EDTA	NaF/K_3EDTA commercial tube	2
2	LH	Lithium heparin commercial tube	4
3	LH/GLY [10]	12–30 IU of Lithium heparin and 160 mmol/L of GLY	4
4	LH/MAN [15]	12–30 IU of and 8.4 mmol/L of MAN	4
5	LH/GLY/ MAN	12–30 IU of Lithium heparin, 160 mmol/L of GLY and 8.4 mmol/L of MAN	4

Note: NaF; NaF/K_3EDTA; Sodium fluoride/tri-potassium EDTA tube (Commercial tube), LH; Lithium heparin tube (commercial tube), LH/GLY; 12–30 IU of lithium heparin/160 mmol/L of GLY, LH/MAN; 12–30 IU of lithium heparin/8.4 mmol/L of mannose, LH/GLY/MAN; LH 12–30 IU/160 mmol/L of GLY/8.4 mmol/L of mannose.

2.4. Blood specimen collection and sample preparations

This study was approved by the Human Research Ethics Committee (approval no. P3-0093/2564) from the Naresuan University Committee, Thailand. Participants comprised 20 healthy subjects and 20 subjects with diabetes. The participants' ages ranged from 20 to 65 y. All the subjects had fasted for 12 h before the scheduled appointments and were seated for 15 min before blood collection.

Whole blood samples (18 ml) were collected aseptically by venipuncture, filled into five labelled aliquots and then mixed thoroughly by inverting each tube ten times. The five blood samples were then transferred separately into 1.5 mL microtubes. Each microtube was centrifuged at 3500 rpm for 10 min. After centrifugation, the microtubes were stored at a constant room temperature of 25 ± 2 °C for 0–8 h before plasma separation was initiated. All plasma samples were carefully stored at −40 °C until measurement (Fig. S1).

2.5. Glucose and biochemical determinations

All plasma samples were measured for glucose, electrolytes, and biochemical tests. To avoid variability, all samples were analyzed within 2 h of thawing in the same batch (baseline results). The glucose and 14 biochemical tests were performed in triplicate using a Cobas c111 automated analyzer (Roche Diagnostics, GmbH Mannheim, Germany) with the manufacturer's reagents, calibrator, and control materials at room temperature (25 ± 2 °C).

2.6. Data analysis

Plasma glucose obtained from an NaF/K_3EDTA tube was used as experimental control. Plasma glucose levels were measured in the blood samples treated with various concentrations of antiglycolytic inhibitors, at time points 0, 2, 4, 6, and 8 h at room temperature (25 ± 2 °C), were then calculated as percentages of decreasing values from the baseline.

Comparisons among the means of the 14 biochemical tests were analyzed normality by and using analysis of variance (ANOVA) with Tukey's honest significant difference (HSD) test, whereas AST and ALT, the Friedman test was used for non-parametric data. Statistical significance was set at p-value <0.05, $p < 0.01$, and $p < 0.001$.

Bias was calculated by determining the average difference between the measurements taken at the 8-h time points and those taken at baseline. This difference was divided by the measurement at baseline and then multiplied by 100 to represent the bias as a percentage. Johns Hopkins Hospital (JHH) threshold criteria (Table S1) were acceptable clinical limits [18].

3. Results

3.1. Decreases of glucose levels in three combinations

Figs. 1 and 2 showed glucose's means and standard deviations (SDs) and the decreasing percentages of glucose in healthy and DM blood samples. The baseline glucose concentrations did not differ significantly ($p > 0.05$) among the three combinations and the NaF/K_3EDTA control group. The LH/GLY/MAN tubes exhibited the highest preservation of glucose in the blood samples, with a decrease ranging from 6.3 to 6.6%. Similarly, the LH/MAN tubes effectively preserved glucose levels, with a decrease ranging from 7.2 to 8.7%. Both combinations of blood additives-maintained glucose reductions within the acceptable threshold value established by the JHH criteria, demonstrating their efficacy in preserving glucose levels.

3.2. Effect of the two blood additives on the biochemical tests in healthy and DM blood samples

The biases of the 14 routine biochemical tests acquired from LH/GLY/MAN and LH/MAN blood samples were below the JHH criteria when compared to the initial baseline measurements for healthy (Table 3), and diabetic subjects (Table 4) over a span of 8 h. However, specific biochemical assays revealed statistically significant differences in albumin, urea, total bilirubin, and lactate dehydrogenase in both groups, and cholesterol, creatinine, uric acid, potassium, and chloride, particularly in the diabetes group.

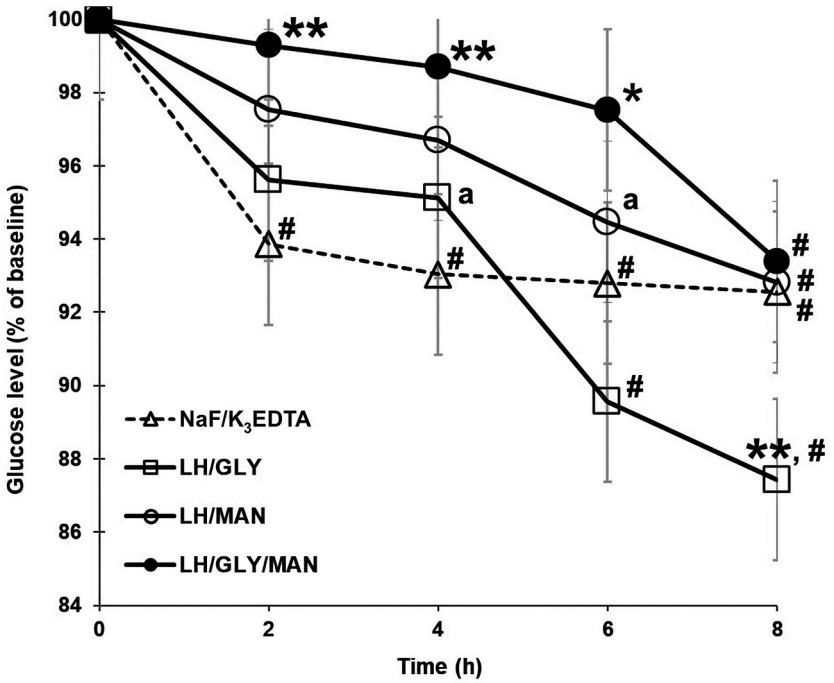

Fig. 1. Percentage decrease in average plasma glucose levels observed in blood mL treated with different antiglycolytic agents over 0–8 among healthy subjects under room temperature conditions.
NaF/K_3EDTA; Sodium fluoride/tri potassium EDTA tube (commercial tube).
LH/GLY; 12–30 IU of lithium heparin/160 mmol/L of GLY.
LH/MAN; 12–30 IU of lithium heparin/8.4 mmol/L of mannose.
LH/GLY/MAN; 12–30 IU of lithium heparin/160 mmol/L of GLY/8.4 mmol/L of mannose.
* Significant difference at $p < 0.05$ when compared to the NaF/K_3EDTA tube at the same time point.
** Significant difference at $p < 0.01$ when compared to NaF/K_3EDTA tube at the same time point.
[a]; Significant difference at $p < 0.05$ when compared to baseline.
[#]; Significant difference at $p < 0.01$ when compared to baseline.

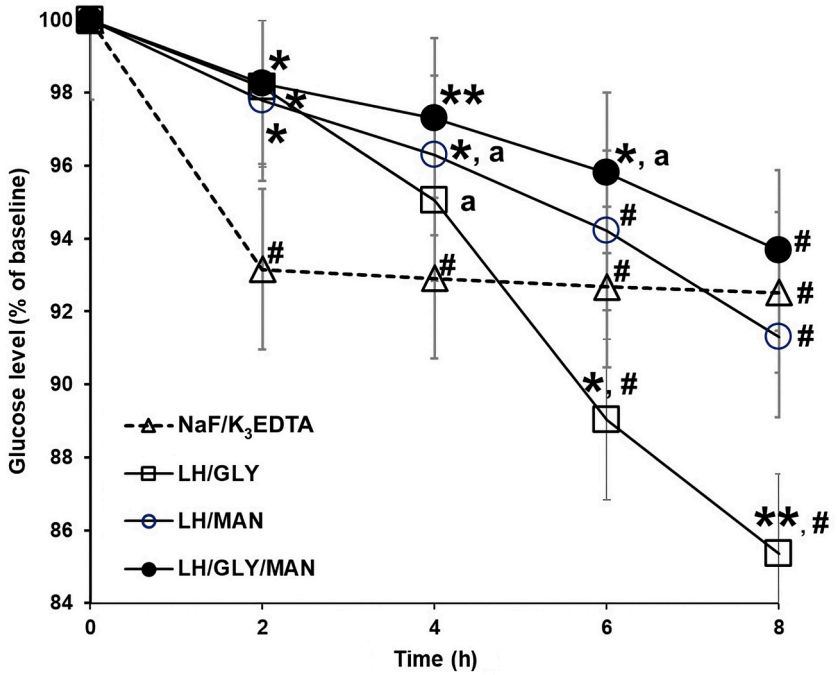

Fig. 2. Percentage decrease in average plasma glucose levels observed in blood treated with different antiglycolytic agents over 0–8 among subject with diabetes mellitus under room temperature conditions.
NaF/K_3EDTA: Sodium fluoride/tri potassium EDTA tube (commercial tube).
LH/GLY; 12–30 IU of lithium heparin/160 mmol/L of GLY.
LH/MAN; 12–30 IU of lithium heparin/8.4 mmol/L of mannose LH/GLY/MAN;
12– 30 IU of lithium heparin/160 mmol/L of GLY/8.4 mmol/L of mannose.
* Significant difference at $p < 0.05$ when compared to the NaF/K_3EDTA tube at the same time point.
** Significant difference at $p < 0.01$ when compared to NaF/K_3EDTA tube at the same time point.
[a] Significant difference at $p < 0.05$ when compared to baseline.
[#] Significant difference at $p < 0.01$ when compared with baseline.

Table 3
Mean (SDs) of 14 biochemical tests derived from heparinized plasma samples treated with different antiglycolytic agents among healthy subjects (n = 20).

Test	JHH Criteria (%)	LH					LH/GLY				
		0 h	4 h		8 h		0 h		4 h		8 h
		Mean (SD)	Mean (SD)	Bias (%)	Mean (SD)	Bias (%)	Mean (SD)	Bias (%)	Mean (SD)	Bias (%)	Mean (SD)
CHOL (mg/dL)	5	104.3 (21.4)	104.0 (21.2)	0.3	103.8 (20.2)	0.5	104.5 (22.2)	0.2	104.0 (24.9)	0.3	103.7 27.1)
TG (mg/dL)	5	99.8 (34.9)	99.6 (33.2)	0.2	99.4 (32.4)	0.4	100.5 (35.4)	0.7	100.2 (36.5)	0.4	99.8 (35.8)
TP (g/dL)	5	7.91 (0.41)	7.91 (0.44)	0.0	7.89 (0.54)	0.3	7.90 (0.40)	0.1	7.86 (0.45)	0.6	7.84 (0.50)
ALB (g/dL)	7	4.94 (0.24)	4.93 (0.23)	0.2	4.92 (0.21)	0.4	4.85 (0.25)	0.8	4.88** (0.20)	1.2	4.85*** (0.26)
UREA (mg/dL)	10	11.7 (2.2)	11.5 (2.0)	1.4	11.5 (2.4)	1.0	11.8 (2.2)	1.2	11.7 (2.3)	0.3	11.4*** (2.4)
CRE (mg/dL)	7	0.86 (0.14)	0.86 (0.14)	0.0	0.85 (0.15)	0.7	0.86 (0.16)	0.0	0.85 (0.15)	1.2	0.87 (0.14)
UA (mg/dL)	10	5.78 (0.85)	5.78 (0.75)	0.0	5.77 (0.87)	0.2	5.75 (0.97)	0.5	5.74 (0.82)	0.6	5.72 (0.79)
AST (U/L)	10	23.2 (7.93)	23.6 (7.93)	1.7	23.9 (7.42)	2.1	22.6 (8.08)	2.6	22.8 (7.49)	1.8	23.1 (7.25)
ALT (U/L)	15	16.7 (11.4–20.4)	16.6 (12.8–17.7)	0.6	16.5 (12.1–18.3)	1.2	16.6 (10.3–17.9)	0.6	16.5 (12.4–16.7)	1.2	16.3 (12.3–19.3)
LDH (U/L)	10	199 (22.4)	199 (29.6)	0.5	200 (25.3)	0.6	198 (28.3)	0.3	199 (28.7)	0.2	201* (22.0)
BIL-T (mg/dL)	10	0.86 (0.45)	0.84 (0.45)	2.3	0.83 (0.42)	3.6	0.85 (0.44)	1.2	0.84 (0.44)	2.3	0.82* (0.46)
Na^+ (mmol/L)	3	137 (1.34)	137 (1.58)	0.1	138 (1.59)	0.3	138 (1.16)	0.1	138 (1.80)	0.1	138 (1.53)
K^+ (mmol/L)	5	3.6 (1.21)	3.7 (1.23)	0.1	3.7 (1.26)	1.1	3.6 (1.23)	0.5	3.7 (1.19)	0.5	3.7 (1.33)
Cl^- (mmol/L)	5	106 (3.02)	106 (2.89)	0.6	106 (3.22)	0.6	106 (3.74)	0.3	106 (2.68)	0.5	105 (3.17)

Abbreviation: LH; Lithium heparin, LH/GLY; 12-30 IU of lithium heparin/160 mmol/L of glyceraldehyde, LH/MAN; 12–30 IU of lithium heparin/8.4 mmol/L of mannose, LH/GLY/MAN; 12–30 IU of lithium heparin/160 mmol/L of glyceraldehyde/8.4 mmol/L of mannose, * Significant difference at $p < 0.05$ when compared to LH at baseline, ** Significant difference at $p < 0.01$ when compared to LH at baseline, *** Significant difference at $p < 0.001$ when compared to LH at baseline.
Footnote: Bias; Mean test - Mean target (LH)/Mean target (LH) ×100 when compared with baseline, ALT was not normally distributed and analyzed with non-parametric test by Friedman test with the medium of 95% confidence intervals and significant value set at $p < 0.05$.

4. Discussion

Glycolysis involves a sequence of enzymatic reactions within cells that convert glucose into pyruvate, generating adenosine triphosphate [19]. NaF is often used in blood collection tubes to inhibit glycolysis by targeting enolase. However, concerns have emerged because of its slow action and potential interference with enzymatic tests [4–6,12]. During the initial stages of glycolysis, glucose undergoes phosphorylation and is transformed into various intermediates through enzymatic reactions. Unlike glucose, MAN is a monosaccharide and a simple sugar that cannot be further broken down. Mannose is not a direct substrate for glycolysis. Studies [20,21] have confirmed that MAN inhibit hexokinase, a primary enzyme that regulates glycolysis. Hexokinase initiates glycolysis by phosphorylating glucose to form glucose-6-phosphate, which is then entrapped within the cell for metabolism. Mannose competes with glucose, binds to the active site of hexokinase, and hinders the conversion of glucose to glucose-6-phosphate. This impedes the glucose entry into the glycolytic pathway (Fig. S2) that modifies from Fig. 1 of Chandel NS [19].

When MAN combines with GLY, it accelerates the action of early glycolytic enzymes, thereby preserving blood glucose levels. This technique is more effective than that of conventional glycolytic inhibitors. The effect of this combination is more extensive and potent. These findings are consistent with those of Nakashima et al. [15], and Mithu et al. [17], who highlighted MAN as a competitive glycolysis inhibitor suitable for maintaining glucose levels in collected blood samples. The inhibition of glucose consumption underscores MAN's preservative role in blood glucose. The research conducted by Chan et al. [16] revealed that MAN effectively maintained blood glucose levels for a minimum of 3 days. It exhibited a slower rate of decline in comparison to heparin-treated samples when kept at room temperature. There was no clinically significant difference in the mean glucose level of the combination of LH, GLY, and MAN from baseline, and it did not with the hexokinase method of measuring glucose levels.

The result of the creatinine assay in this study was different from that of the previous report by Landt [10]; in the earlier study, creatinine levels were determined using the alkaline picrate (Jaffe) method with a Dade Behring RxL-automatic analyzer. The technique was highly susceptible to the positive interference from D- or L-GLY. In contrast, the creatinine results in this study showed no significant interference via the enzymatic method using an automatic analyzer, Cobas c 111 in the DM and healthy groups.

The JHH criteria represent clinical criteria designed to align with the unique demands of medical testing. These criteria establish

LH/GLY	LH/MAN						LH/GLY/MAN					
8 h	0 h		4 h		8 h		0 h		4 h		8 h	
Bias (%)	Mean (SD)	Bias (%)	Mean (SD)	Bias (%)	Mean (SD)	Bias (%)	Mean (SD)	Bias (%)	Mean (SD)	Bias (%)	Mean (SD)	Bias (%)
0.6	103.9 (21.4)	0.4	103.7 (29.2)	0.6	103.5 (29.6)	0.8	104.2 (20.6)	0.1	103.9 (22.9)	0.4	103.5 (22.7)	0.8
0.0	100.1 (32.9)	0.3	99.5 (33.7)	0.3	99.3 (31.5)	0.5	100.2 (33.6)	0.4	99.7 (34.1)	0.1	199.6 (34.0)	0.2
0.9	7.88 (0.42)	0.4	7.88 (0.38)	0.4	7.85 (0.33)	0.8	7.92 (0.33)	0.1	7.90 (0.38)	0.1	7.86 (0.47)	0.6
1.8	4.92 (0.27)	0.4	4.90 (0.22)	0.8	4.89 (0.21)	1.0	4.94 (0.18)	0.0	4.91 (0.19)	0.6	4.90 (0.17)	0.8
2.2	11.7 (2.3)	0.2	11.7 (2.1)	0.3	12.0 (2.3)	2.9	11.6 (2.3)	1.4	11.7 (2.2)	0.5	11.8 (2.1)	0.8
1.2	0.86 (0.14)	0.0	0.86 (0.17)	0.0	0.86 (0.13)	1.15	0.87 (0.14)	1.2	0.86 (0.13)	0.3	0.86 (0.16)	0.2
1.0	5.76 (0.83)	0.3	5.74 (0.86)	0.7	5.72 (0.82)	1.0	5.77 (1.01)	0.1	5.76 (0.98)	0.3	5.73 (0.98)	0.9
0.4	23.2 (8.13)	0.0	23.3 (7.73)	0.4	23.6 (8.12)	1.7	23.6 (7.60)	1.7	23.3 (7.24)	0.4	23.0 (8.11)	0.9
2.4	16.5 (14.4–19.2)	1.1	16.3 (10.6–18.2)	2.3	16.3 (10.0–16.7)	2.5	16.7 (12.5–19.6)	0.1	16.5 (12.4–18.3)	1.2	16.3 (12.3–19.3)	2.3
0.7	199.4 (25.1)	0.3	200 (24.3)	0.6	200.2 (23.8)	0.7	198.6 (24.3)	0.1	199.3 (27.8)	0.3	200 (26.2)	0.6
3.7	0.84 (0.48)	2.8	0.83 (0.42)	3.3	0.83 (0.46)	3.4	0.85 (0.44)	1.2	0.84 (0.45)	2.3	0.83 (0.41)	3.0
0.3	137 (1.41)	0.1	137 (1.50)	0.1	138 (1.42)	0.3	138 (1.53)	0.1	138 (1.38)	0.2	138 (1.43)	0.2
1.1	3.7 (1.33)	0.3	3.7 (1.33)	1.1	3.7 (1.33)	1.1	3.7 (1.23)	0.3	37 (1.27)	0.8	3.7 (1.23)	1.6
0.7	105 (3.27)	0.2	106 (3.27)	0.5	106 (3.27)	0.2	106 (2.87)	0.5	106 (2.62)	0.3	10 (2.89)	0.5

predetermined and clinically meaningful acceptable limits for a variety of measurements. By factoring in the practical implications of measurement deviations within a medical context, these criteria provide a robust assessment grounded in the clinical perspective. Ensuring the accuracy of laboratory test results was achieved by applying the JHH criteria (Tables 3, 4, S1) instead of utilizing ANOVA. The JHH criteria are meticulously tailored clinical standards designed to precisely align with the unique demands of medical testing. These criteria establish predetermined and clinically meaningful acceptable limits for various measurements.

The findings revealed that the combination of LH with GLY and MAN was a suitable composition for glycolytic inhibitory effects and was highly capable of inhibiting glycolysis by preserving the initial glucose concentration for 8 h. NaF/K_3EDTA could only be used in blood glucose assays. These combinations demonstrated the advantages of blood collections and accuracies of 14 routine biochemical tests derived from a single blood sample tube, a process that benefits DM management.

A limitation of this study is that these blood additives, LH/GLY/MAN and LH/MAN, need to be further investigated once they are included in blood collection tubes. In this study, these additives were only introduced for 14 biochemical tests together with glucose, but there are several biochemical tests that need to be further investigated. Such investigations could potentially improve the applicability of these additives in medical laboratories, contribute to more accurate routine tests and increase the accuracy of blood analyzers.

We developed a LH blood additive comprising a combination of MAN, GLY and that ensures glucose stability and avoids clinically significant biochemical measurements. An optimal preservation of glucose levels in blood samples was achieved by combining LH, 8.4 mmol/L of MAN, and 160 mmol/L of GLY. This preservative ensured glucose stability without disrupting the results of 14 routine biochemical tests, even when the samples were stored at room temperature for up to 8 h. With further studies, this approach can contribute to more precise routine tests and improve the accuracy of blood analysis instruments.

Research funding

This work was supported by the Program Management Unit for Competitiveness Enhancement (PMU-C) 2021 (grant number C10F640049).

Contribution

RW, KM and WT: Research, wrote the manuscript. GJK: Edited the manuscript. AC, NA: Provided expert feedback.

CRediT authorship contribution statement

Renu Wiriyaprasit: Formal analysis, Methodology, Writing – original draft. **Khundaw Moonla:** Data curation, Validation, Writing – review & editing. **Napaporn Apiratmateekul:** Investigation, Supervision. **Anchalee Chittamma:** Investigation, Supervision. **Gerald J. Kost:** Supervision, Writing – review & editing. **Wanvisa Treebuphachatsakul:** Funding acquisition, Project administration, Supervision, Validation, Writing – review & editing.

Table 4
Mean (standard deviations) of 14 biochemical tests derived from heparinized plasma samples treated with different antiglycolytic agents among those with diabetes mellitus (n = 20).

Test	JHH Criteria (%)	LH					LH/GLY				
		0 h	4 h		8 h		0 h		4 h		8 h
		Mean (SD)	Mean (SD)	Bias (%)	Mean (SD)	Bias (%)	Mean (SD)	Bias (%)	Mean (SD)	Bias (%)	Mean (SD)
CHOL (mg/dL)	5	207 (23.1)	207 (24.4)	0.1	205 (24.2)	1.0	205 (23.6)	1.0	204 (21.7)	1.4	199*** (24.4)
TG (mg/dL)	5	167 (32.7)	166 (32.1)	0.5	166 (31.3)	0.4	167 (32.1)	0.1	167 (32.6)	0.0	167 (39.1)
TP (g/dL)	5	7.74 (0.64)	7.68 (0.63)	0.8	7.66 (0.54)	1.0	7.68 (0.66)	0.8	7.66 (0.64)	1.0	7.65 (0.69)
ALB (g/dL)	7	4.28 (0.17)	4.27 (0.33)	0.2	4.25 (0.20)	0.7	4.25 (0.27)	0.7	4.24 (0.24)	0.9	4.22** (0.24)
UREA (mg/dL)	10	18.8 (6.3)	19.0 (6.3)	0.8	18.9 (6.1)	0.3	18.8 (6.1)	0.2	18.7 (6.4)	0.9	18.4*** (6.5)
CRE (mg/dL)	7	0.91 (0.13)	0.90 (0.13)	1.1	0.89** (0.16)	2.2	0.90 (0.17)	1.1	0.88** (0.15)	3.3	0.86*** (0.13)
UA (mg/dL)	10	6.9 (1.80)	6.8 (1.91)	0.3	6.9 (1.80)	0.1	6.9 (1.75)	0.1	6.8 (1.78)	0.3	6.8*** (1.79)
AST (U/L)	10	21.6 (19.0–23.6)	21.5 (17.0–22.9)	0.5	22.3 (16.0–21.5)	3.2	21.4 (15.5–20.9)	0.9	21.9 (15.5–20.9)	1.4	22.2 (15.9–21.5)
ALT (U/L)	15	12.6 (10.6–16.9)	12.1 (6.9–15.8)	4.0	11.7 (5.8–14.3)	7.1	12.3 (6.5–14.4)	2.4	11.8 (6.5–15.1)	6.3	11.7 (6.9–14.9)
LDH (U/L)	10	205 (21.2)	205 (22.3)	0.2	206 (20.2)	0.7	204 (19.8)	0.2	206 (19.5)	0.7	209*** (17.7)
BIL-T (mg/dL)	10	0.45 (0.18)	0.43 (0.15)	4.4	0.42** (0.13)	6.7	0.46 (0.12)	2.2	0.45 (0.12)	0.0	0.44 (0.11)
Na^+ (mmol/L)	3	140 (2.3)	139 (2.5)	0.3	139 (2.2)	0.8	140 (2.6)	0.2	139 (2.5)	0.4	140 (2.7)
K^+ (mmol/L)	5	4.4 (0.58)	4.4 (0.76)	0.5	4.3 (0.77)	1.4	4.4 (0.61)	0.5	4.4 (0.68)	1.1	4.2** (0.70)
Cl^- (mmol/L)	5	104 (3.9)	104 (3.7)	0.2	104 (4.1)	0.5	104 (3.7)	0.2	104 (4.3)	0.5	103** (3.6)

Abbreviation: LH; Lithium heparin, LH/GLY; 12–30 IU of lithium heparin/160 mmol/L of glyceraldehyde, LH/MAN; 12–30 IU of lithium heparin/8.4 mmol/L of mannose, LH/GLY/MAN; 12–30 IU of lithium heparin/160 mmol/L of glyceraldehyde/8.4 mmol/L of mannose, * Significant difference at $p < 0.05$ when compared to LH at baseline, ** Significant difference at $p < 0.01$ when compared to LH at baseline, *** Significant difference at $p < 0.001$ when compared to LH at baseline.
Footnote: Bias; Mean test - Mean target (LH)/Mean target (LH) ×100 when compared with baseline, AST and ALT were not normally distributed and analyzed with non-parametric test by Friedman test with the medium of 95% confidence intervals and significant value set at $p < 0.05$.

LH/GLY	LH/MAN						LH/GLY/MAN					
8 h	0 h		4 h		8 h		0 h		4 h		8 h	
Bias (%)	Mean (SD)	Bias (%)	Mean (SD)	Bias (%)	Mean (SD)	Bias (%)	Mean (SD)	Bias (%)	Mean (SD)	Bias (%)	Mean (SD)	Bias (%)
4.0	209 (22.4)	0.8	206 (24.2)	0.7	203* (21.9)	1.8	207 (25.4)	0.2	206 (25.7)	0.2	207 (22.8)	0.3
0.2	168 (36.1)	0.6	167 (36.9)	0.1	166 (36.7)	0.4	167 (34.4)	0.1	167 (33.5)	0.1	167 (39.7)	0.3
1.2	7.75 (0.65)	0.1	7.75 (0.49)	0.1	7.73 (0.51)	0.1	7.76 (0.5)	0.3	7.66 (0.55)	0.0	7.64 (0.62)	1.0
1.4	4.3 (0.29)	0.5	4.29 (0.29)	0.2	4.26 (0.27)	0.5	4.29 (0.23)	0.2	4.26 (0.18)	0.2	4.11 (0.23)	0.5
2.3	18.6 (5.8)	1.3	18.5* (5.6)	1.8	18.3** (5.7)	2.9	18.7 (6.1)	0.7	18.5 (6.2)	1.3	18.5 (6.1)	1.8
5.5	0.91 (0.14)	0.0	0.91 (0.17)	0.0	0.90 (0.13)	1.1	0.91 (0.16)	0.0	0.90 (0.11)	1.1	0.89 (0.16)	1.1
1.3	6.8 (1.73)	0.3	6.8 (1.76)	0.4	6.7*** (1.82)	2.2	6.85 (1.87)	0.1	6.83 (1.80)	0.3	6.8 (1.80)	0.4
2.8	21.8 (18.1–23.1)	0.9	22.1 (17.3–23.4)	2.3	22.4 (18.5–23.1)	3.7	21.4 (17.5–3.4)	0.9	22.2 (17.1–23.6)	2.3	22.5 (16.8–23.7)	2.8
7.1	11.8 (10.4–17.2)	6.3	11.7 (8.6–14.2)	7.1	11.5 (7.0–14.7)	8.7	12.9 (8.9–18.9)	2.4	10.8 (7.6–16.6)	0.8	11.5 (6.6–15.5)	6.3
2.3	206 (15.1)	0.7	206 (14.3)	0.6	205 (13.8)	0.4	205 (14.1)	0.3	206 (17.8)	0.7	208 (16.8)	0.8
2.2	0.45 (0.12)	0.0	0.44 (0.15)	2.2	0.43 (0.13)	4.4	0.46 (0.13)	2.2	0.44 (0.14)	0.0	0.42 (0.12)	2.2
0.1	140 (2.7)	0.4	139 (2.7)	0.6	138* (2.7)	0.7	140 (2.4)	0.2	140 (2.7)	0.4	143 (2.2)	0.3
2.0	4.4 (0.52)	0.5	4.3 (0.52)	1.4	4.3 (0.52)	1.6	4.4 (0.62)	0.5	4.4 (0.67)	0.2	4.5 (0.66)	1.1
1.1	104 (4.2)	0.1	104 (4.2)	0.4	103* (4.2)	0.6	104 (3.5)	0.1	104 (3.1)	0.3	104.9 (3.1)	0.4

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plabm.2024.e00388.

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