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The evidence for clinically significant bias in plasma glucose between liquid and lyophilized citrate buffer additive

Gordana Juricic [a,*], Andrea Saracevic [b], Lara Milevoj Kopcinovic [b], Ana Bakliza [c], Ana-Maria Simundic [d]

[a] Department of Laboratory Diagnostics, General Hospital Pula, Pula, Croatia
[b] University Department of Chemistry, University Hospital Centre "Sestre Milosrdnice", Zagreb, Croatia
[c] Department of Laboratory Diagnostics, Psychiatric Hospital "Sv. Ivan", Zagreb, Croatia
[d] Department of Medical Laboratory Diagnostics, University Hospital "Sveti Duh", Zagreb, Croatia

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ABSTRACT

Objectives: Citrate buffer additive has been suggested to be of supreme performance in inhibiting glycolysis. However, there is little evidence in the literature regarding the comparability of glucose concentrations in liquid and lyophilized citrate buffer containing tubes. The aim of this study was to compare glucose concentrations in tubes containing liquid (Glucomedics) and lyophilized citrate buffer (Terumo VENOSAFE™ Glycemia) additive, measured immediately after centrifugation.

Design and methods: Blood was collected from forty volunteers into both Glucomedics and Venosafe Glycemia tubes. Blood was centrifuged within 15 min from venipuncture and glucose concentration was measured immediately after centrifugation, on the Abbott Architect analyzer. Differences between glucose concentrations in Glucomedics and Terumo tubes were tested using the paired *t*-test. Mean bias was calculated and compared to recommended quality specification for glucose (i.e. 2.2%).

Results: Glucose concentration in Terumo tubes was 3.4% lower than in Glucomedics tubes ($P < 0.001$). The mean bias was clinically significant.

Conclusions: There is a clinically significant difference between glucose concentrations in liquid and lyophilized citrate buffer additive tubes (Glucomedics vs. Terumo tubes) measured immediately after centrifugation. This difference may affect the patient outcome due to the misclassification of diabetes.

1. Introduction

Tube components and tube additives are known to have a potential to alter test results [1]. Their use should therefore be validated and carefully considered prior to implementing into the routine everyday practice. Citrate buffer was introduced few years ago into practice as a new, superior antiglycolytic agent, and was implemented in the American Diabetes Association (ADA) and the National Academy of Clinical Biochemistry (NACB) guidelines for laboratory analysis in the diagnosis and management of diabetes mellitus (DM) [2]. According to these guidelines, the samples for glucose determination should be drawn either in tubes containing citrate buffer, or alternatively tubes should be placed in ice-water slurry immediately after sampling and plasma separated within 30 min. This would imply the comparability of obtained glucose results independently of the tube type used, as long as sample handling is carried out as recommended.

Commercially available citrate buffer additives are available either in lyophilized or liquid form, depending on the manufacturer. Our group has already demonstrated that liquid citrate acidification is effective in glycolysis inhibition, even during prolonged storage in un-centrifuged tubes [3,4]. Many other authors have also been able to demonstrate the effect of citrate acidification [5–9].

Whether there is a difference in performance between liquid and lyophilized additive has not yet been properly investigated. Two independent studies have investigated the stability of glucose concentration in un-centrifuged whole blood samples collected in tubes containing lyophilized (Terumo VENOSAFE™ Glycemia) and liquid (Sarstedt GlucoEXACT) citrate buffer additive [10,11]. Comparable glucose stability for up to 48 h has been demonstrated, irrespective of the type of citrate buffer additive used. However, even though these studies investigated and compared the long term stability of glucose concentration in these tubes, their limitation is the absence of direct comparison of glucose values obtained immediately after venipuncture in both tube types [10,11].

The potential advantage of the liquid additive is its immediate and uniform homogenization with whole blood, whereas the introduction of a dilution factor derived from components such as hematocrit and fill volume might be regarded as a potential disadvantage and a source

Abbreviations: ADA, American Diabetes Association; NACB, National Academy of Clinical Biochemistry; DM, diabetes mellitus.

* Corresponding author at: Department of Laboratory Diagnostics, General Hospital Pula, Aldo Negri 6, Pula, Croatia.
E-mail address: gjuricic@gmail.com (G. Juricic).

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of bias in glucose results. With the use of lyophilized additive, the risk of dilution bias is avoided. Nevertheless, the use of lyophilized additive increases the risk of sample hemolysis and subsequent appearance of an unusual brownish coloration of the plasma samples as demonstrated in a study on Terumo VENOSAFE™ Glycemia tubes [12].

Terumo has discontinued its production of blood collection tubes, including Glycaemia tubes containing citrate buffer additive, with the end of 2015. These tubes were the first and until now the only commercially available tubes containing lyophilized citrate buffer additive since its introduction as the additive of choice for glucose determination. To the best of our knowledge, only two additional manufacturers (i.e. Greiner Bio-One and Sarstedt) provide citrate buffer additive tubes but exclusively in liquid form. Whereas VENOSAFE™ Glycemia tubes were available worldwide, Greiner Bio-One's Glucomedics tubes are currently available in West and Central Europe and Russia and are not available on the US market.

When Terumo has discontinued the production of their tubes, laboratories which have been using the VENOSAFE™ Glycemia tubes were forced to shift from lyophilized to liquid citrate buffer additive. However, whether different forms of citrate buffer additive are equally effective in glycolysis inhibition has not been investigated so far.

The aim of this study was therefore to explore whether glucose concentrations measured from tubes containing citrate buffered additive in liquid and lyophilized form are comparable. To fulfill this aim, we have compared glucose concentrations measured in tubes containing liquid (Glucomedics, Greiner BioOne) and lyophilized citrate buffer (Terumo VENOSAFE™ Glycemia) additive.

2. Materials and methods

2.1. Study design

Forty non-fasting healthy volunteers aged 33 (23–60) years were recruited from November 2014 to January 2015 at the Clinical Institute of Chemistry, University Hospital Center "Sestre Milosrdnice" (Zagreb, Croatia). Blood was collected in the non-fasting state, between 12 and 4 p.m. to ensure that a broader glucose measurement range is covered. An informed consent was signed by all participants. The study was approved by the institutional Ethics Committee and conducted according to the principles of the Helsinki Declaration. Blood collection was performed according to the recommendations of Croatian Society of Medical Biochemistry and Laboratory Medicine [13]. To minimize venipuncture bias, all venipunctures were performed by a single experienced phlebotomist.

Blood was collected from the antecubital vein using a Vacuette Multiple Use Draw, 21 gauge needles, Greiner Bio-One (Kremsmuenster, Austria; Ref. No. 450076). Samples were collected into 2 types of tubes:

1) Glucomedics sodium EDTA/sodium fluoride/citric acid/sodium citrate tube, 2 mL, Ref. No. 454347 (Glucomedics) — additive in liquid form, and
2) Terumo VENOSAFE™ Glycemia EDTA/sodium fluoride/citrate buffer tube, 2 mL, Ref. No. VF-052SFC (Terumo) — additive in lyophilized form.

To minimize collection order bias, the order of draw was randomized. All samples were handled according to manufacturer's instructions [14–16].

After venipuncture, all tubes were carefully mixed by gently inverting the tubes 8–10 times. All samples were centrifuged within 15 min from venipuncture at 1800 *g* for 10 min in the Rotixa 50 RS centrifuge (Hettich Lab Technology, Tuttlingen, Germany) at 22 °C. Glucose measurements were performed from all tubes immediately after centrifugation on the Architect c8000 chemistry analyzer (Abbott Diagnostics, Chicago, USA) using the hexokinase method (within-laboratory CV = 1.55%). Glucose concentrations measured in Glucomedics tubes were multiplied by 1.16 as recommended by the manufacturer.

2.2. Statistical analysis

The data sets were tested for normality using the Kolmogorov–Smirnov test. Since all data were distributed normally, the results were presented as mean ± standard deviation (SD). The differences between glucose concentrations measured in Glucomedics and Terumo tubes were tested using the paired *t*-test. To test for clinically significant difference, the biases between each tube pair were calculated using the following equation: $B = [(Glu_{Terumo} - Glu_{Glucomedics}) / Glu_{Glucomedics}] \times 100$. The mean bias was then compared to ADA recommendation for glucose quality specification (i.e. 2.2%) [2].

In addition, to assess the agreement between glucose concentrations measured in Glucomedics and Terumo tubes, Bland–Altman and Passing–Bablok analyses were done. Statistical analysis was performed using the MedCalc statistical software, version 12.5.0 (Ostend, Belgium). Values of $P < 0.05$ were considered statistically significant.

3. Results

Mean glucose concentrations measured in Glucomedics and Terumo tubes are presented in Table 1. The glucose concentration range measured in Glucomedics and Terumo tubes was 4.7–8.7 mmol/L and 4.7–8.4 mmol/L, respectively. Glucose concentrations measured in Terumo tubes were lower than those measured in Glucomedics tubes ($P < 0.001$). The mean bias between Terumo and Glucomedics tubes (−3.4%) exceeded the predefined ADA acceptance criteria [2] and was considered clinically significant. Although Passing–Bablok regression analysis did reveal neither systematic nor proportional difference, Bland–Altman analysis has demonstrated a constant bias between the two tube types, as presented in Fig. 1.

4. Discussion

The key finding of our study is the existence of a substantial and clinically significant difference in plasma glucose measurements respective to the type of citrate additive used (liquid or lyophilized). Glucose concentration measured in Terumo tubes is 3.4% lower than in Glucomedics tubes. The clinically significant bias found between the Glucomedics and Terumo tubes can not only introduce confusion into laboratory results interpretation, but also lead to misdiagnosis of DM and inadequate patient management. The results of our study are particularly important for laboratories forced to replace Terumo tubes with tubes containing citrate additive in liquid form.

The comparison of tubes with citrate buffer additive has been the focus of many research groups in the past few years. Brief summary of studies which have performed similar comparisons in the past (citrate buffer tubes vs. NaF/KOx or lithium-heparin tubes), as well as the key findings from these studies are summarized in Table 2. Interestingly, all studies investigating Terumo tubes [5,6,8,9] with lyophilized citrate buffer additive, found no significant bias between these tubes and NaF/KOx or lithium-heparin tubes processed immediately after venipuncture. Conversely, studies investigating Glucomedics tubes manufactured by Greiner Bio-One and containing liquid citrate buffer additive found a clinically significant bias between the tested tubes [3,4,7]. A possible cause for such a discrepancy in results reported in the

Table 1
Mean glucose concentrations and mean bias determined in Glucomedics and Terumo tubes.

Tube type	Glucose, mmol/L	P*	Mean bias, (%)	Recommended ADA criteria (%)
Glucomedics Terumo	6.0 ± 1.0 5.8 ± 0.9	<0.001	−3.4	±2.2

Data were presented as mean ± standard deviation. ADA — American Diabetes Association. * Statistical difference tested using the paired *t*-test. $P < 0.05$ was considered statistically significant.

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