Incomplete filling of lithium heparin tubes affects the activity of creatine kinase and γ-glutamyltransferase

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ABSTRACT

This study aims to assess whether or not incomplete filling of primary lithium heparin tubes may influence the activity of creatine kinase (CK), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and γ-glutamyltransferase (GGT). Blood was drawn from 20 healthy volunteer using an identical sequence of tubes. First, a 6 mL, 13 x 100 mm 14 unit/mL lithium heparin Vacuette was filled and discharged. Then, three identical lithium heparin Vacuette tubes were filled, one to the nominal volume (i.e., full-draw tube), another with half of the nominal volume (half-draw tube) and the last with one-third of the nominal volume (low-draw tube). The plasma was separated and tested for CK (non-activated by *N*-acetylcysteine), AST and ALT on a Beckman Coulter Unicel DxC 800. Tests for CK were performed with a different reagent on a Beckman Coulter AU5800 (activated by *N*-acetylcysteine). Although the concentrations of ASL and ALT measured on the Unicel DxC and that of CK measured on the AU5800 did not change significantly across the different specimens, those of CK and GGT measured on the Unicel DxC 800 were significantly increased in the half-draw and low-draw tubes. The percentage bias of CK on the Unicel DxC 800 (using Bland Altman plots) was 3.3% and 7.9% for the half-draw and low-draw tubes, respectively, whereas that of GGT was 10.3% and 16.6% for the half-draw and low-draw tubes, respectively. These results suggest that short-draw lithium heparin tubes might be unsuitable for testing GGT and CK using specific combinations of reagents and instrumentation.

KEY WORDS: Blood specimen collection.
Creatine kinase.
Heparin.
Interference.

Introduction

Laboratory data contribute substantially to the clinical decision-making and management of a variety of human disorders. Nevertheless, laboratory information can only be useful when a high degree of quality is established throughout the total testing process,[1] from sample collection[2] to result(s) reporting.[3]

Several lines of evidence now suggest that the manually intensive activities of the pre-analytical phase represent the most vulnerable and critical steps of the testing process, especially those directly related to collection and handling of biological specimens.[4,5] The receipt of insufficient specimens is not so rare in clinical laboratories, with a prevalence as high as 0.7 per 1000 (i.e., 9–21% of all unsuitable specimens). The collection of partially filled blood tubes is commonplace, especially for coagulation tubes, with an estimated prevalence as high as 15–30% of all routine samples.[6,7]

The recent Clinical and Laboratory Standards Institute (CLSI) H18-A3 guideline on procedures for handling and processing blood specimens emphasises that partial drawing of lithium heparin primary blood tubes might determine a significant bias in the measurement of plasma creatine kinase (CK), so that the excess amount of additive has the potential to adversely affect the accuracy of test results.[8]

This recommendation is based on an article published by Andrejat *et al.* in 1982,[9] which reported that the concentration of CK measured on a Rotochem IIa (Travenol Instrument Division, Jessup MD, USA) with Beckman substrate was dramatically influenced by the heparin concentration in the test sample. In particular, the CK activities of the plasma samples were 205 U/L in samples containing 48 units/mL heparin and 219 U/L in those containing 72 units/mL heparin, versus 185 U/L in samples containing no heparin. Thus, it was concluded that plasma CK values might be significantly higher in the presence of the high heparin concentrations that may result when heparinised tubes are incompletely filled.

In the 30 years since publication of this report, new instrumentation, new formulations of reagent and especially new vacuum blood tubes made of different materials and containing a lower heparin concentration are now available on the market.[10] Therefore, this study aims to assess whether or not the incomplete filling of primary lithium heparin tubes might influence the activity of CK, as well as that of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and γ-glutamyltransferase (GGT).

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Materials and methods

The study population consisted of 20 healthy volunteer (7 males, 13 females; mean age 45 years [range: 32–59]) recruited from laboratory personnel. Blood was drawn from a peripheral vein of the arm, by the same experienced

phlebotomist (GL), using a 20 G blood collection needle with Luer adapter (Greiner Bio-One, Kremsmünster, Austria). An identical sequence of tubes was used for each subject.

First, a 6 mL, 13 x 100 mm 14 unit/mL lithium heparin Vacuette (Greiner Bio-One) was completely filled and discharged after collection (i.e., the discard tube); then, a second identical lithium heparin Vacuette was completely filled to the nominal volume (i.e., full-draw tube), a third identical lithium heparin Vacuette was filled to half the nominal volume (half-draw tube), and a fourth identical lithium heparin Vacuette was filled to one-third of the nominal volume (low-draw tube).

All steps of sample collection were standardised, including the time the tourniquet was in place (<20 sec), the use of needles and tubes of the same lot number, and mixing of blood and additive (i.e., by gentle inversion [x6] of the tube immediately after collection). No specimen was discarded for unsatisfactory or challenging venipuncture.

The plasma was immediately separated by centrifugation (1300 xg) for 10 min at room temperature, and tested in duplicate for CK (CK for Synchron Lx and UniCel DxC systems), AST (enzymatic rate method), ALT (kinetic rate method) and GGT (γ-glutamyl-ρ-nitroaniline enzymatic rate assay) on a Beckman Coulter Unicel DxC 800 (Beckman Coulter, Brea CA, USA), and CK with a different reagent on a Beckman Coulter AU5800 (i.e., CK-Nac for the AU series). Both CK assays are enzymatic rate methods, but the latter is a modification of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) reference assay,[11] and differs in composition and final concentration of reagents (i.e., AU5800 reagent contains imidazole [pH 6.5] and *N*-acetylcisteine). Lithium heparin is considered a suitable anticoagulant for both assays and no interference is reported up to a final heparin concentration of 29 units/mL.

The within-run imprecision (expressed in terms of the coefficient of variation [CV%]) quoted by the manufacturer is 0.6–4.3% for ATL on the Unicel DxC 800, 0.6–4.2% for AST on the Unicel DxC 800, 0.5–12.0% for GGT on the Unicel DxC 800, 0.8–3.0% for CK on the Unicel DxC 800, and 1.0–1.5% for CK on the Beckman Coulter AU5800, respectively.

The volunteers provided informed consent and the study was carried out in accordance with the Declaration of Helsinki and under the terms of all relevant local legislation. Statistical analysis was performed with Analyse-it for Microsoft Excel (Analyse-it Software, Leeds, UK), and included the Wilcoxon/Mann-Whitney test to assess the differences between paired specimens, and Bland Altman plots to define the bias. Results of testing were expressed as mean and standard error of the mean (SEM), whereas the bias was reported as mean and 95% confidence interval (95% CI). The clinical significance of variations was also assessed against the desirable specifications of the bias derived from intra- and inter-individual biological variation.[12]

Results

The blood collected in the first sample (i.e., full-draw tube) was 6.0 mL, whereas the mean (±SEM) amount of blood collected in the second and third samples was 3.0±0.1 (half-draw tube) and 2.1±0.1 mL (low-draw tube), resulting in a final concentration of lithium heparin in the samples of 14.3

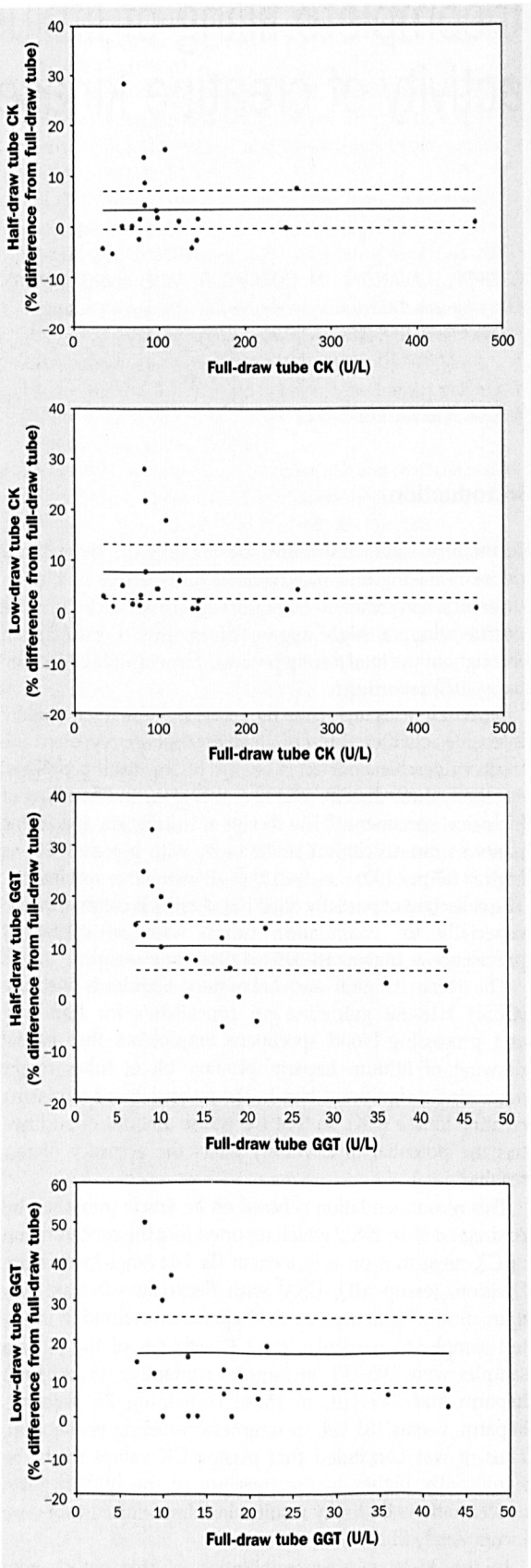

Fig. 1. Bland Altman plots of the difference between measurements of creatine kinase measured on the Unicel DxC (CK-DxC) and γ-glutamyltransferase (GGT) in full-draw, half-draw and low-draw lithium heparin tubes. Solid lines are drawn at the mean difference; dashed lines define the 95% confidence interval (95% CI).

Table 1. Variation of plasma enzymes according to the filling volume of primary vacuum lithium heparin tubes.

	Lithium heparin tube		
	Full-draw	Half-draw	Low-draw
Volume (mL)	6.0±0.0	3.0±0.1	2.1±0.1
Heparin (u/mL)	14.3±0.0	29.0±0.8	40.6±0.9
CK-AU (U/L)	126±22	126±22	127±22
CK-DxC (U/L)	125±22	128±23*	132±22*
AST (U/L)	22±1	23±1	23±2
ALT (U/L)	27±3	27±3	26±3
GGT (U/L)	17±2	19±3*	19±2*

Results shown as mean and standard error of the mean (SEM).

CK-AU: creatine kinase measured on AU5800; CK-DxC: creatine kinase measured on Unicel DxC; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: γ-glutamyltransferase.

*$P<0.05$ versus full-draw tube.

units/mL in the full-draw tubes, 29.0±0.8 units/mL in the half-draw tubes and 40.6±0.9 units/mL in the low-draw tubes. Interestingly, while the concentrations of ASL and ALT measured on the Unicel DxC and that of CK measured on the AU5800 did not change significantly across the different specimens, those of CK and GGT measured on the Unicel DxC 800 were significantly increased in the half-draw and low-draw tubes (Table 1).

The percentage bias of CK on the Unicel DxC 800 (Bland Altman plots) was 3.3% (95%CI, -0.5–7.0%) for the half-draw tubes and 7.9% (95% CI, 2.6–13.2%) for the low-draw tubes, and were within the desirable specifications of the bias derived from intra- and inter-individual biological variation (i.e., ±11.5%).[12] Conversely, the percentage bias of GGT for the half-draw tubes was 10.3% (95% CI, 5.5–15.0%) and 16.6% (95% CI, 7.4–25.7%) for the low-draw tubes, which exceeded the desirable specifications of the bias in the latter sampling condition (i.e., ±10.8%) (Fig. 1).[12]

Discussion

Lithium heparin is the preferred additive for plasma clinical chemistry testing, and the nominal amount of dry heparin in primary vacuum tubes is typically 14.3 units/mL blood.[10] Although three salts of heparin are commercially available (i.e., lithium, sodium and ammonium), the first is the most widely used because the use of sodium heparin may determine an over-estimation of sodium concentration, whereas ammonium heparin may generate a spurious increase in urea nitrogen.[10] Some differences in test results of certain clinical chemistry parameters have been described between serum and lithium heparin plasma, and mostly involve increased potassium and lower total protein concentrations in lithium heparin plasma.[10] However, no significant differences have been reported for plasma enzymes such as AST, ALT, CK and GGT between serum and lithium heparin plasma.[10]

According to the current CLSI H18-A3 guideline, short-draw lithium heparin tubes should be avoided when measuring the plasma activity of CK, as the presence of excess heparin may jeopardise the chemical reaction.[8] This recommendation is based on an earlier study by Andrejat *et al*,[9] who reported that the reaction rate of CK was faster in samples with higher heparin concentration in the first 120 sec after the lag-phase.

This faster rate has mainly been observed with the Beckman substrate, characterised by a lower pH (6.1) such as that of CK for the Synchron Lx and UniCel DxC systems, but not with other substrates that show a higher pH (6.5; i.e., that of the CK-Nac on the AU series), and has been reliably attributed to precipitation of β-lipoproteins as induced by heparin and Mg^{2+} in the substrate at pH ~6.0.[9]

The results of the present investigation are in agreement with these findings and further confirm that the traditional Beckman Coulter reagent for Unicel DxC systems overestimates CK in low-draw heparin plasma. A mean bias of 7.9% was seen in samples containing heparin ~40 units/mL, whereas the bias observed by Andrejat and co-authors was 11% in two plasma samples containing heparin at 48 units/mL. However, the new reagent for AU series systems, which contains imidazole to stabilise the pH (6.5), is virtually unaffected by the final concentration of lithium heparin in the specimen, so that test results might be reliable even in short-draw lithium heparin tubes. It is, however, noteworthy that the bias observed measuring CK activity on the Unicel DxC 800 did not achieve clinical significance when compared with the current desirable specifications of the bias derived from intra- and inter-individual biological variation, neither in the half-draw nor in the low-draw tubes.[12]

With regard to GGT, there has been no report of a bias in inappropriately filled lithium heparin samples. The only study that showed any interference was published by Dimeskia and Carter in 2005, and they described the case of two samples that contained IgM paraproteins, which produced interference with the Roche GGT method when collected as lithium heparin plasma samples. In one sample the GGT activity was 127 U/L in lithium heparin plasma versus 77 U/L in serum.[13] The bias could also be reproduced by adding heparin to the serum specimens and was eliminated by addition of a heparin antagonist (i.e., protamine sulphate), thereby confirming that the interference was caused by heparin.

The present results suggest that the interference in GGT testing due to excess heparin in the specimen (all the samples tested were negative for paraproteins) may also be present using other reagent formulation and instrumentation. Although these results should be verified using different tubes, reagents and instrumentation, it is concluded that short-draw lithium heparin tubes might be unsuitable for testing CK and especially GGT. □

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