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## SHORT COMMUNICATION

# High-Sensitivity Cardiac Troponin T: a Preanalytical Evaluation

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## SUMMARY

***Background:*** **Little is known about the effects of preanalytical conditions on high-sensitivity cardiac troponin T (hs-cTnT) concentrations.**
***Methods:*** **Variations of hs-cTnT concentrations were evaluated under the following preanalytical conditions: 1) serum vs. lithium-heparin (Li-Hep) plasma, with or without separator gel; 2) centrifugation time (15-minutes vs. 10-minutes) and speed (1467 to 3756 g); 3) stability in Li-Hep plasma at room temperature and +4°C for 4 days and at -80°C for up to 12 months, for three concentrations; 4) four freeze-thaw cycles at -20°C and -80°C, for three concentrations.**
***Results:*** **No significant changes were found regarding the type of blood collection tube, the centrifugation, and storage conditions. Minor decreases were observed after four freeze-thaw cycles at -20°C (< 6.5%) and -80°C (< 3.4%).**
***Conclusions:*** **High-sensitivity cardiac troponin T may be considered as not impacted by usual preanalytical conditions, thus strengthening its reliability in laboratory practice and clinical research.**
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high-sensitivity cardiac troponin T, preanalytical, centrifugation, stability

## INTRODUCTION

Currently, high-sensitivity cardiac troponin T (hs-cTnT) is one of the main biomarkers used in emergency and routine laboratory for detection of acute myocardial infarction (AMI) in early phase of pain onset [1]. Hs-cTnT has been widely studied, on the diagnostic and prognostic plans [2-4]. The 20 and 50% increases after 3 to 6 hours have been proposed as significant variations for rapid rule-in/-out of AMI [5], depending on the initial result. However, according to the reference change value (RCV) approach, an AMI should be suspected if the relative hs-cTnT change exceeds the sum of the variations influencing its concentration, which are mainly analytical and biological (within- and inter-indi-

vidual) [6]. The impact of interferences and analytical performances of the hs-cTnT electrochemiluminescent immunoassay (ECLIA) have been evaluated [7-9], but little is known about the effects of usual preanalytical treatments on this biomarker. These should be considered since they may potentially participate to the global variance of results [6]. Depending on the hs-cTnT-based studies, preanalytical conditions may differ substantially: blood samples may be collected into serum tubes with separator gel [10], or into lithium heparin plasma tubes with separator gel [11], or may be centrifuged at 3000 g [11] or at 2000 g [12]. Furthermore, in clinical trials, plasma samples may be stored at -20°C or -70°C until assays [13]. These examples illustrate the diversity of practices in laboratory regarding the type of blood collection tubes, centrifugation or storage conditions, which may impact hs-cTnT results, but are totally unheeded in clinical trials.
In order to answer and enrich such issues, we aimed to test the impact of the following preanalytical conditions on hs-cTnT results: type of blood collection tube, centrifugation time and speed, storage stability depending on time and temperature, and freeze-thaw cycles effect.

## MATERIALS AND METHODS

Plasma and serum hs-cTnT concentrations were measured by ECLIA on a Modular E170® analyzer (Roche Diagnostics) [14], characterized by intra-assay imprecisions of 2.62%, 2.02%, 1.19%, and 2.17% for 5.85, 18.8, 83.5, and 532 ng/L mean concentrations, respectively, and inter-assay imprecisions of 2.40% and 1.58% for 28.15 and 2176 ng/L mean concentrations, respectively, as determined from quality control values accumulated over a 22-month period (PreciControl Troponin®; Roche Diagnostics).

**1) Comparison serum versus plasma**
Hs-cTnT concentrations were measured on serum (S) and plasma from heparinized tubes with (HG) and without separator gel (H), collected simultaneously from 44 hospitalized patients. Blood collection tubes used were serum tubes from Becton Dickinson (BD Vacutainer™, Serum tube, CAT, ref #369032) and lithium heparin tubes from Greiner Bio-one™ (18 U/mL lithium heparin, separator gel, ref #474080; and without gel, ref #474084).

**2) Effect of centrifugation time and speed**
The comparison of 15-minute vs. 10-minute centrifugation time was assessed for 90 pairs of heparin tubes sampled from hospitalized patients: the first tube was centrifuged at 1885 g for 15 minutes on a Modular Preanalytics® (MPA) thermostated (17.5 ± 2.5°C), and the second one was simultaneously centrifuged at 1885 g for 10 minutes on a Rotina 380-R® centrifuge (Hettich, Germany) at 17°C.
The comparison of centrifugation speeds was assessed for 15 heparin tubes. Plasma hs-cTnT concentrations were sequentially measured after a 1467, 2113, 2876, and 3756 g centrifugation speed (i.e., 2500, 3000, 3500 and 4000 rpm), using the Rotina 380-R® centrifuge, with a 5-minute re-homogenization on a circular rotator between each centrifugation.

**3) Stability in heparin plasma**
Firstly, hs-cTnT stability in heparin plasma from three level pools was daily assessed during a 4-day storage at RT and +4°C. During this period, all the HG tubes were kept in vertical position and immediately recapped after measurement on the analyzer.
Secondly, middle- and long-term stability at -80°C was tested for three heparin plasma pools. The frozen aliquots were thawed for reassessment after 7, 14, and 30 days (middle-term stability). Three others level pools were assayed after 3, 9, and 12 months of storage at -80°C (long-term stability).

**4) Stability after freeze-thaw cycles**
Aliquots from three heparin plasma pools were stored at -20°C and -80°C, in order to test the hs-cTnT stability after four freeze-thaw cycles. During the four days following their preparation, daily and at the same time, the three pools were thawed at RT during one hour, briefly centrifuged at 1885g for 5 minutes to discard fibrin, and then analyzed before being refrozen. Each day, all these steps were performed within a total thaw delay of two hours.

**Calculations and Statistics**
Data were analysed using Microsoft Excel® and MedCalc® (MedCalc, Belgium) software. Comparisons were assessed using Passing-Bablok and Bland-Altman tests, and Cusum test for linearity. Inter-group comparisons were done using a non-parametric unpaired test (Kruskal-Wallis test) or repeated measures analysis of variance (ANOVA test). Statistical significance was defined as $p < 0.05$.

## RESULTS

**1) Comparison serum versus plasma**
The three comparisons showed a highly linear relationship, and concentrations did not differ statistically ($p = 0.989$, Figure 1; Passing-Bablok and Bland-Altman graphs in supplemental Figure 1A-F). On the measuring ranges, mean biases (-1.96 SD; +1.96 SD) were: S vs. H: +2.1% (-7.1; +11.3%); S vs. HG: +2.0% (-7.5; +11.5%); and HG vs. H: +0.1% (-7.8; +8.1%).

**2) Effect of centrifugation time and speed**
The hs-cTnT concentrations did not change significantly whether plasma was centrifuged for 15 or 10 minutes (mean bias +2.1% (-9.1; +11.5%); supplemental Figure 2A, B), nor using different centrifugation speeds ($p = 0.964$, supplemental Figure 2C).

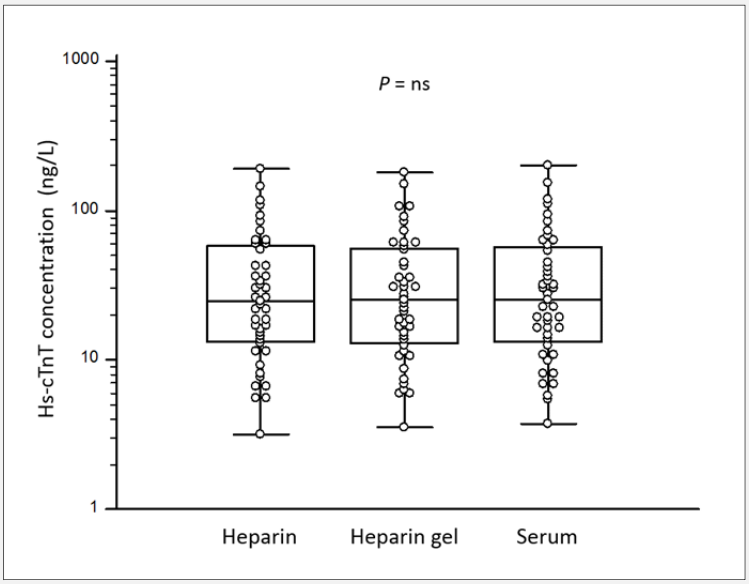



**Figure 1. Influence of the type of blood collection tube on high-sensitivity cardiac troponin T concentrations.**

**Legend: Box and whisker plot for hs-cTnT concentrations assayed in supernatants from centrifuged heparin tubes, heparin gel tubes and dry tubes from 44 hospitalized patients. Abbreviations: hs-cTnT - high-sensitivity cardiac troponin T, ns - non-significant.**

**3) Stability in heparin plasma**

As shown in Figure 2A, the hs-cTnT concentrations remained stable up to 4 days storage at RT, for low, medium, and high levels pools (-2.6 to +0.4% change from baseline) and at +4°C (-3.2 to +0.8% change of baseline). When stored at -80°C (Figure 2B), plasma hs-cTnT concentrations can be considered as stable up to 30 days (-2.2 to +4.9% change from baseline), even up to 12 months at -80°C, except for the lower level of hs-cTnT (-12.6 to +8.5%).

**4) Stability after freeze-thaw cycles**

Whatever the hs-cTnT concentrations, a maximum decrease from about 6.5% and 3.5% from baseline occurred over four successive freeze-thaw cycles at -20°C and -80°C, respectively (Figure 3).

## DISCUSSION

Most of the conditions tested here were without significant impact on hs-cTnT concentrations. Concerning the type of blood collection tube, we did not find any significant difference between hs-cTnT concentrations measured in serum or in lithium heparin plasma, with or without gel separator, in accordance with the results of Gillis et al. [15]. For hs-cTnT assays, plasma should be preferred to serum, since it avoids the clotting time (~30 minutes) required for complete separation of serum, allowing a shortened turnaround time and thus saving time when AMI is suspected. Hence, our results confirmed that heparin plasma can be used preferably for hs-cTnT measurement. According to laboratory practices, centrifugation time and speed may differ substantially [11,12]. Our study pointed out that hs-cTnT concentration did not vary significantly depending on centrifugation time and speed, thus confirming previous results from Minder et al. [16]. In routine laboratory practice, centrifuged tube and/or plasma aliquots may have to be re-assayed for hs-cTnT measurement, in case of omission from prescriber, lab recording error, need for result confirmation, or other causes, thus justifying storage of plasma for hours or days, usually at 2 - 8°C. Furthermore, in clinical research, hs-cTnT measurements are generally performed on frozen plasma samples, sometimes stored for months. Gillis et al. neither found any significant change in heparin plasma hs-cTnT concentrations after storage for up to 24 hours at RT or at 2 - 8°C, nor over 3 months at -80°C [15]. Mansour et al. did not observe significant hs-cTnT variation in heparin plasma refrigerated at 2 - 8°C nor when measured daily over one week [17]. Confirming and reinforcing such results, we did not observe significant changes in hs-cTnT concentrations for up to 4 days of storage at RT or +4°C, nor over one year at -80°C. We did not evaluate the stability at -20°C as did Gillis et al. who

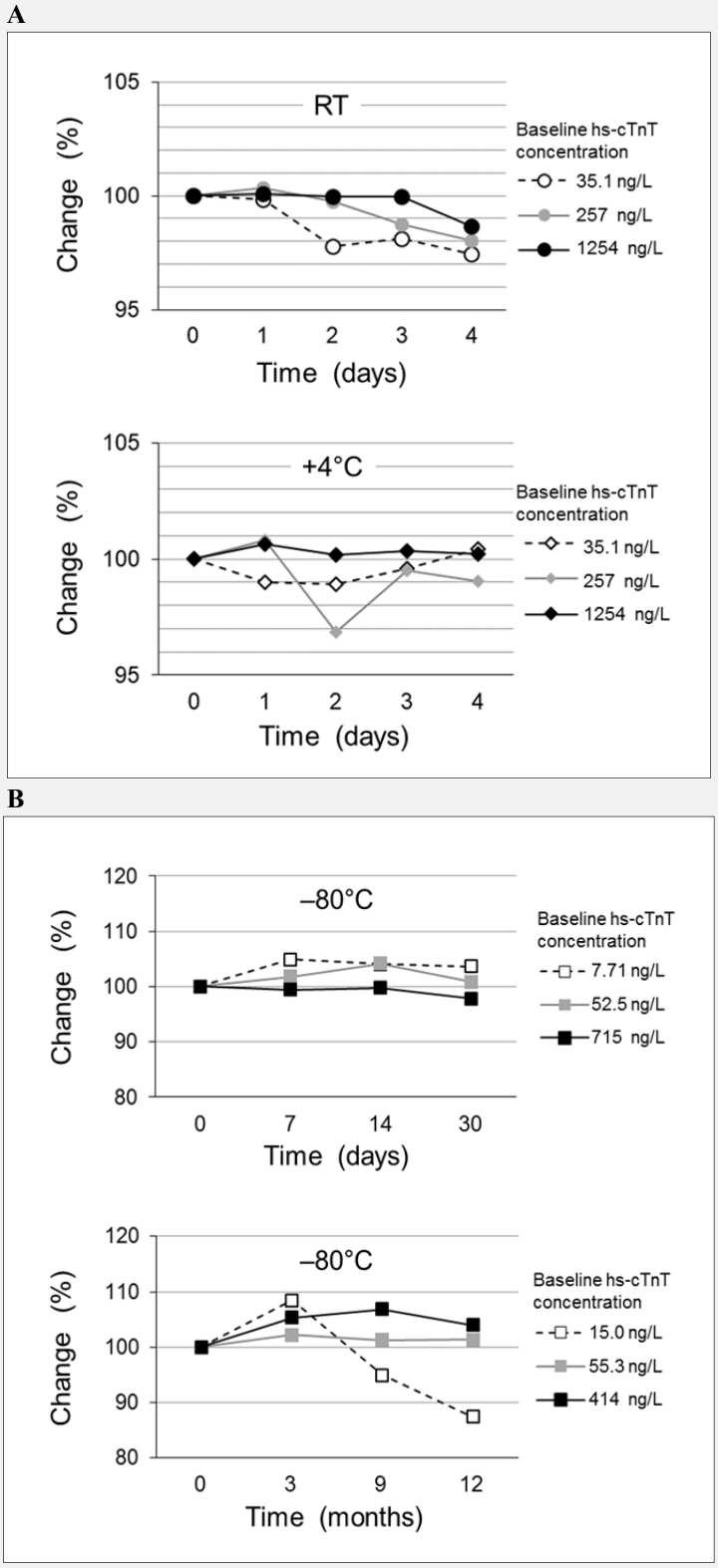



**Figure 2. Stability of heparin plasma high-sensitivity cardiac troponin T depending on temperature and time of storage.**

**Legend: Dot and line graphs of short-term stability over a 4-day storage at RT and +4°C (A), middle-term stability up to 30 days, and long-term stability up 12 months at -80°C (B), for three hs-cTnT concentration pools. Abbreviations: hs-cTnT - high-sensitivity cardiac troponin T, RT - room temperature.**

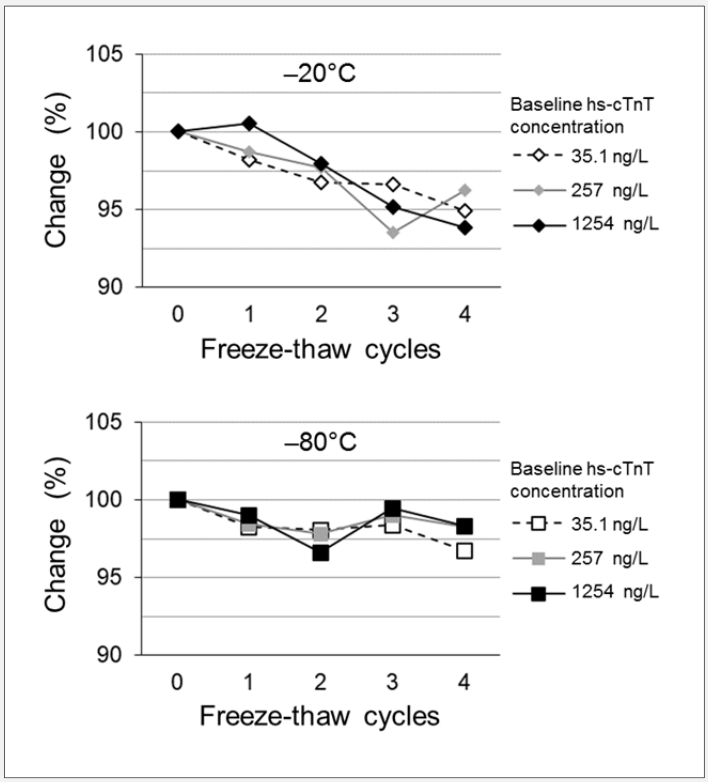



**Figure 3. Influence of freeze-thaw cycles at -20°C and -80°C on heparin plasma high-sensitivity cardiac troponin T concentrations.**

**Legend: Dot and line graph of percent change of hs-cTnT concentrations after four freeze-thaw cycles, at -20 and -80°C, for three pool levels. Abbreviations: hs-cTnT - high-sensitivity cardiac troponin T.**

showed a high stability for up to 3 months at this temperature [15]. However, we showed that plasma hs-cTnT concentration remains stable after up to 2 freeze-thaw cycles when stored at -20°C, and up to 4 cycles when stored at -80°C, in accordance with the results of Mansour et al. showing that hs-cTnT concentration remained stable after 3 freeze-thaw cycles in EDTA plasma [17]. In some multicentre studies, patients' samples may have to be first frozen at -20°C in each centre (because of unavailable -80°C freezer), then delivered to a central reference laboratory and frozen at -70 to -80°C until hs-cTnT assays [13]. Our results show that such organizational strategy can be safely conducted without any significant impact on hs-cTnT results.

## CONCLUSION

The present preanalytical study shows that hs-cTnT can be indifferently assayed in serum or lithium heparin plasma with or without gel separator and is not impacted by centrifugation time and speed. Heparin plasma hs-cTnT can be considered as stable when stored at room temperature and +4°C for up to 4 days, at -80°C for up one year and remains stable after 2 freeze-thaw cycles at -20°C and 4 cycles at -80°C. All these results strengthen the reliability and robustness of hs-cTnT in laboratory practice and clinical research.

**Declaration of Interest:**
None.

**References:**

1. Thygesen K, Alpert JS, Jaffe AS, et al. Third universal definition of myocardial infarction. J Am Coll Cardiol 2012;60:1581-98.

2. Giannitsis E, Becker M, Kurz K, Hess G, Zdunek D, Katus HA. High-sensitivity cardiac troponin T for early prediction of evolving non-ST-segment elevation myocardial infarction in patients with suspected acute coronary syndrome and negative troponin results on admission. Clin Chem 2010;56:642-50.

3. Weber M, Bazzino O, Navarro Estrada JL, et al. Improved diagnostic and prognostic performance of a new high-sensitive troponin T assay in patients with acute coronary syndrome. Am Heart J 2011;162:81-8.

4. Saunders JT, Nambi V, de Lemos JA, et al. Cardiac troponin T measured by a highly sensitive assay predicts coronary heart disease, heart failure, and mortality in the Atherosclerosis Risk in Communities Study. Circulation 2011;123:1367-76.

5. Thygesen K, Mair J, Giannitsis E, et al. How to use high-sensitivity cardiac troponins in acute cardiac care. Eur Heart J 2012;33: 2252-7.

6. Ricós C, Cava F, García-Lario JV, et al. The reference change value: a proposal to interpret laboratory reports in serial testing based on biological variation. Scand J Clin Lab Invest 2004;64: 175-184.

7. Giannitsis E, Kurz K, Hallermayer K, Jarausch J, Jaffe AS, Katus HA. Analytical validation of a high-sensitivity cardiac troponin T assay. Clin Chem 2010;56:254-61.

8. Chenevier-Gobeaux C, Meune C, Blanc MC, Cynober L, Jaffray P, Lefevre G. Analytical evaluation of a high-sensitivity troponin T assay and its clinical assessment in acute coronary syndrome. Ann Clin Biochem 2011;48:452-8.

9. Bais R. The effect of sample hemolysis on cardiac troponin I and T assays. Clin Chem 2010;56:1357-9.

10. Torbicki A, Kurzyna M, Kuca P, et al. Detectable serum cardiac troponin T as a marker of poor prognosis among patients with chronic precapillary pulmonary hypertension. Circulation 2003; 108:844-8.

11. Hickman PE, McGill D, Potter JM, Koerbin G, Apple FS, Talaulikar G. Multiple biomarkers including cardiac troponins T and I measured by high-sensitivity assays, as predictors of long-term mortality in patients with chronic renal failure who underwent dialysis. Am J Cardiol 2015;115:1601-6.

12. Adams KF Jr, Mehra MR, Oren RM, et al.Prospective evaluation of the association between cardiac troponin T and markers of disturbed erythropoiesis in patients with heart failure. Am Heart J 2010;160:1142-8.

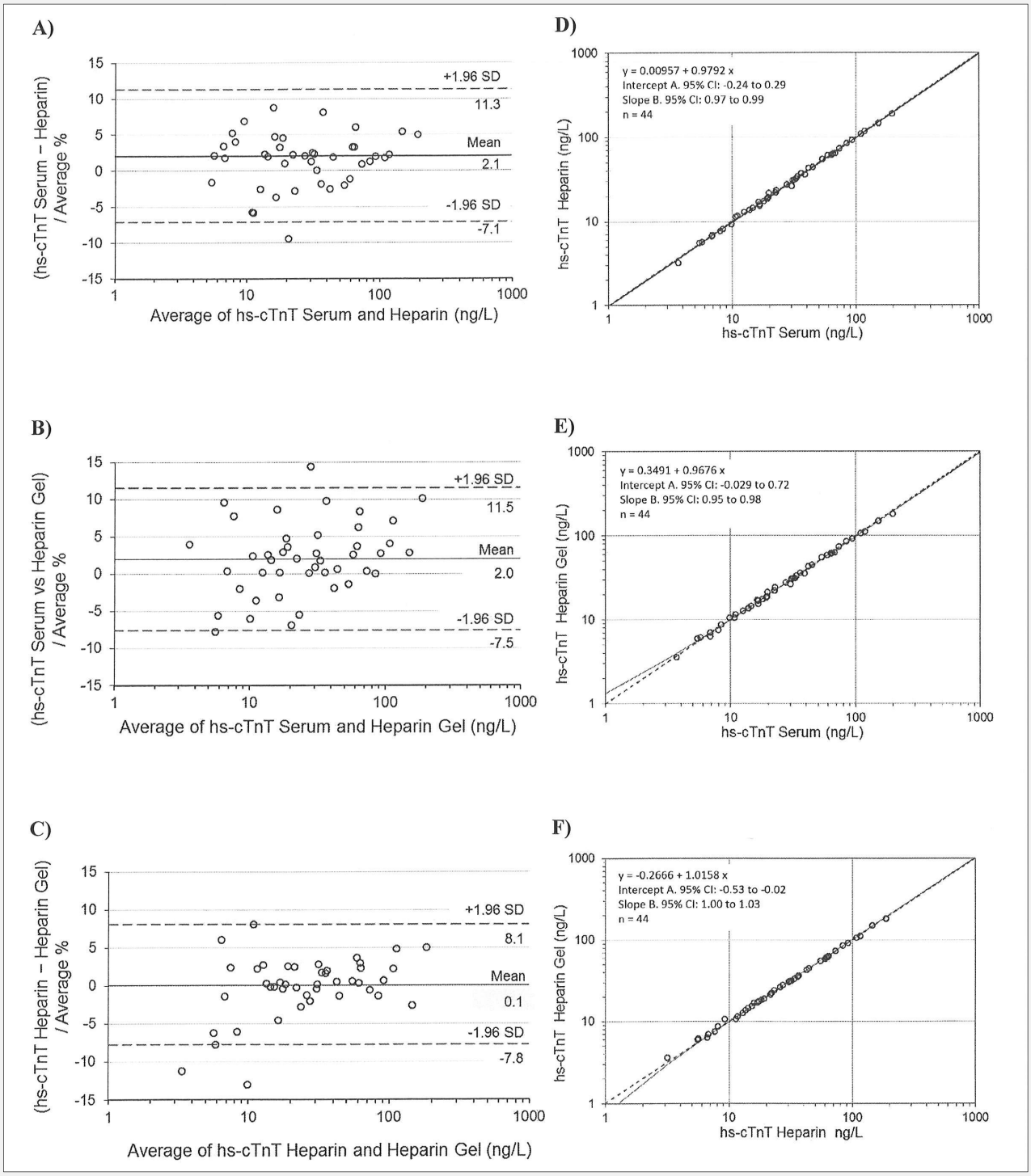
13. Omland T, de Lemos JA, Sabatine MS, et al. A sensitive cardiac troponin T assay in stable coronary artery disease. N Engl J Med 2009;361:2538-47.

14. Monneret D, Abbes RA, Omarjee R, et al. Analytical comparison of the new point-of-care troponin T immunoassay on AQT90-Flex® analyzer (Radiometer) and the high-sensitivity troponin T immunoassay on Modular E170® (Roche Diagnostics). Clin Chem Lab Med 2014;52:e279-82.

15. Gillis JM, Dunselman P, Jarausch J, de Jong N, Cobbaert CM. Preanalytical storage does not affect 99th percentile cardiac troponin T concentrations measured with a high-sensitivity assay. Clin Chem 2013;59:442-3.

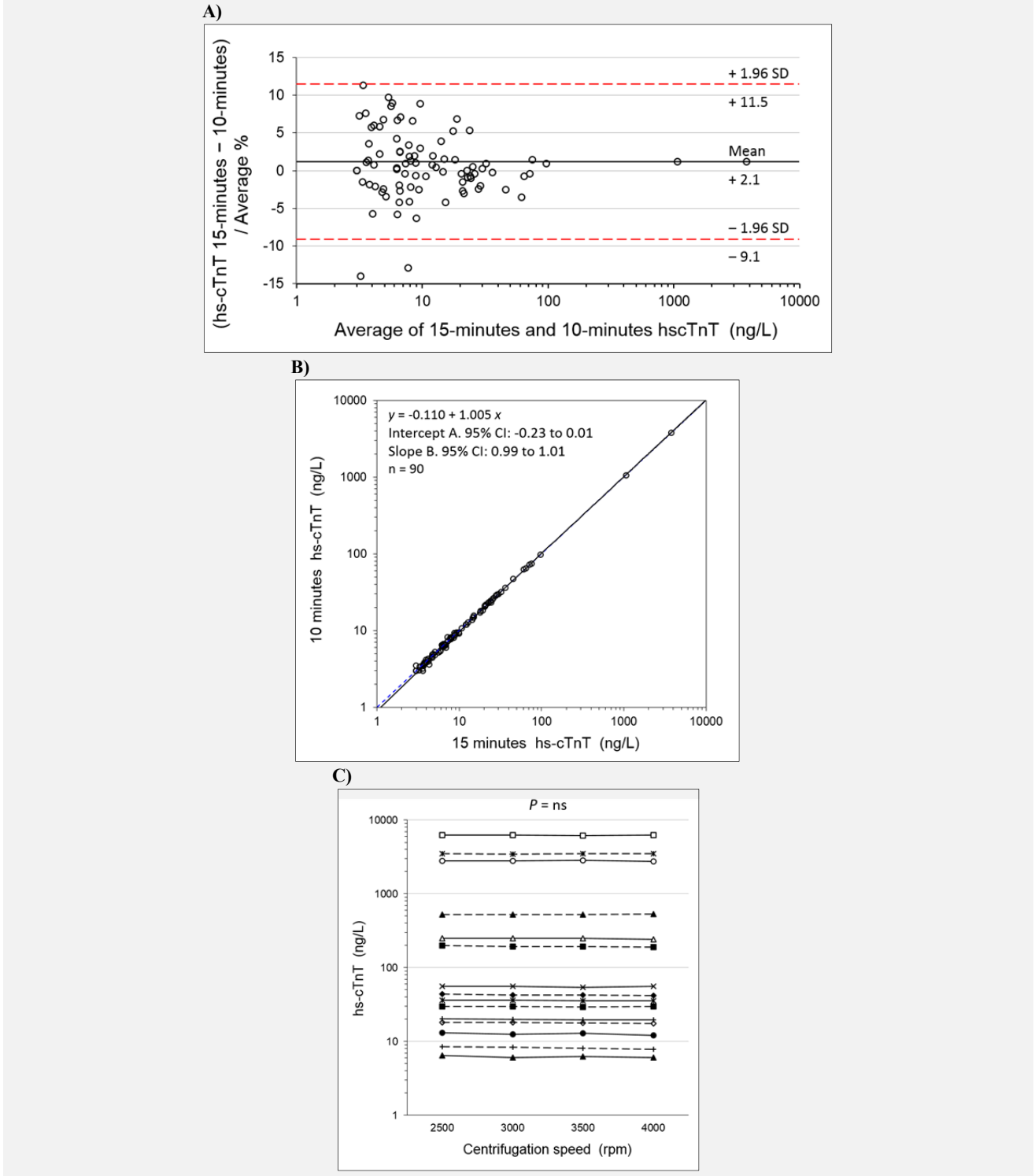
16. Minder EI, Schibli A, Mahrer D, Nesic P, Plüer K. Effects of different centrifugation conditions on clinical chemistry and immunology test results. BMC Clin Pathol 2011;11:6.

17. Mansour M, Clark L, Kavsak PA. Effect of freeze-thaw and refrigeration conditions on high-sensitivity troponin T concentrations. Ann Clin Biochem 2012;49:101-2.




**Supplemental Figure 1. Influence of the type of blood collection tube on high-sensitivity cardiac troponin T concentrations.**

**Legend: Bland-Altman plot for hs-cTnT concentrations assayed in serum and heparin tubes (A), in serum and heparin gel tubes (B), and in heparin tubes with and without gel (C). The solid horizontal line denotes the mean difference (%) between hs-cTnT concentrations. The two dotted lines denote the ± 1.96 standard deviations of the difference. Passing-Bablok regression analysis of hs-cTnT concentration assayed in serum and heparin tubes (D), in serum and heparin gel tubes (E), and in heparin with and without gel tubes (F). The dashed line with a slope of 1 corresponds to equivalence and the solid line is the linear regression line.**
**Abbreviations: hs-cTnT- high-sensitivity cardiac troponin T, SD - standard deviation, 95% CI - 95% confidence interval.**




**Supplemental Figure 2. Influence of 15 - versus 10-minute centrifugation time (A, B) and centrifugation speed (C) on heparin plasma high-sensitivity troponin T concentrations.**

**Legend: Bland-Altman plot for hs-cTnT concentrations assayed in heparin tubes after 15 - versus 10-minute centrifugation time (A). The solid horizontal line denotes the mean difference (%) between hs-cTnT concentrations. The two dotted lines denote the ± 1.96 standard deviations of the difference. Passing-Bablok regression analysis of hs-cTnT concentration assayed in heparin tubes after 15 - versus 10-minute centrifugation time (B). The dashed line with a slope of 1 corresponds to equivalence and the solid line is the linear regression line. Dot and line graph of centrifugation speed comparison for heparin plasma hs-cTnT concentrations (C). Abbreviations: hs-cTnT - high-sensitivity cardiac troponin T, ns - non-significant, rpm - rotation per minute, SD - standard deviation, 95% CI - 95% confidence interval.**