Preanalytical Storage Does Not Affect 99th Percentile Cardiac Troponin T Concentrations Measured with a High-Sensitivity Assay

To the Editor:

On the occasion of the introduction of the high-sensitivity cardiac troponin T (hs-cTnT)¹ (fifth-generation) assay from Roche Diagnostics and considering the importance of a stable 99th percentile upper reference limit for early diagnosis of acute myocardial infarction (1), we evaluated the impact of various preanalytical storage conditions on cTnT concentrations around the 99th percentile (14 ng/L). The stability of cTnT as measured with the fifth-generation hs-cTnT assay has previously been shown (2, 3); however, the storage conditions and cTnT concentrations evaluated in these studies were limited.

In this study, we investigated the most common routine storage conditions for whole blood, serum, and heparin-treated plasma. We obtained samples from 90 patients admitted to the catheterization laboratory for suspected minor myocardial damage. We used multiple samples from individual patients instead of pooled serum to prevent masking of possible matrix effects and to study a wider range of cTnT concentrations. Approval was given by the Medical Ethical Committee of Amphia Hospital, Breda, the Netherlands. All subjects gave written informed consent.

For evaluating the effect of storage of whole blood, we collected venous blood from 30 participants into 3 serum tubes each (10-mL Venosafe™ Plastic Tubes, Serum Gel; Terumo) and 3 lithium heparin–treated tubes each (10-mL Venosafe™ Plastic Tubes: Plasma Gel (containing lithium heparin); Terumo). Whole blood was either processed immediately to obtain the fresh frozen (FF) reference or stored at room temperature for 3 h and 6 h. After the specified storage time, primary tubes were centrifuged (2500g for 5 min at 4 °C); the serum or plasma supernatants were then aliquoted into screw cap tubes (conical 2.0-mL cryovials; BöPlopcetics) and immediately frozen at −80 °C. Frozen aliquots from each patient were measured in duplicate in a single batch later in time, with the same calibration curve. More than 540 measurements were performed, calibrator and control samples excluded.

To study the effect of storage of serum and heparin-containing plasma on cTnT concentrations, we collected venous blood from an additional 30 participants into 5 serum tubes and from 30 other participants into 5 lithium heparin–containing tubes. Serum or plasma was obtained from each sample by centrifuging, pooling, and aliquoting within 45 min of blood collection. One aliquot was immediately stored at −80 °C (i.e., FF reference), and the rest of the serum/plasma samples were distributed into separate vials for storage, as follows: at room temperature for 1 h, 2 h, 5 h, and 24 h; at 2 °C–8 °C for 1 h, 2 h, 5 h, and 24 h; at −20 °C without defrost cycle for 24 h, 1 week, 4 weeks, and 3 months; and at −80 °C for 3 months. At the end of each time and condition of storage, the aliquot was stored in the −80 °C freezer. Samples for each of the 14 storage conditions were measured in duplicate in a single batch after about 3 months. We made >1740 measurements, calibrator and control samples excluded.

We calculated the relative difference between the results obtained for each sample at the various storage conditions and those obtained for the FF reference samples. Kruskal–Wallis analysis was used to determine whether any differences between the different conditions were statistically significant (P = 0.05).

We detected no significant differences in cTnT concentrations between the different storage conditions of whole blood, either with anticoagulant (P = 0.978; n = 29 for the interval 4–933 ng/L; median, 12 ng/L) or without anticoagulant (P = 1.000; n = 29 for the interval 4–930 ng/L; median, 13 ng/L) (data not shown). Our collection of a sample into a serum tube and into a heparin-containing tube for each patient in this study arm allowed comparison of cTnT results obtained for serum and heparin plasma samples from the same patient. Consistent with the documented performance of the fourth-generation cTnT assay (in which heparin interference was eliminated) (4), we detected no differences between serum and heparin-containing plasma samples in the cTnT results (n = 87, data not shown). We also observed no significant differences in cTnT results with respect to the 14 storage conditions for serum (P = 1.000; n = 30 for the interval 4–402 ng/L; median, 8 ng/L) and heparin-containing plasma (P = 1.000; n = 28 for the interval 4–451 ng/L; median, 17 ng/L). Fig. 1 summarizes the results obtained for the plasma-storage conditions. The plot shows the mean relative difference ± 2 SDs between results obtained for the different storage conditions and those obtained for the FF reference samples for the 28 patient samples with detectable cTnT.

We conclude that hs-cTnT results around the 99th percentile obtained with the Roche assay

¹ Nonstandard abbreviations: hs-cTnT, high-sensitivity cardiac troponin T (assay); FF, fresh frozen.
are not affected by preanalytical variation produced by prolonged transport and/or storage of whole blood, serum, or heparin-containing plasma. Our findings underscore the robustness of the Roche fifth-generation hs-cTnT assay around the 99th percentile and extend the previously published data on the stability of cTnT in vitro (2, 3, 5).

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References


