Changes in Concentration of DNA in Serum and Plasma during Storage of Blood Samples

To the Editor:

Cell-free DNA in serum and plasma has been suggested to have diagnostic potential because associations between DNA concentrations and several disorders have been described (1). The concentration of cell-free DNA circulating in plasma and serum has been analyzed in several studies and used as an interchangeable index of the quantity of circulating DNA in blood (2–4). However, it is known that the DNA concentration in serum is 3- to 24-fold higher than in plasma (3–6). Recently published articles in this journal showed that various preanalytical factors of blood sampling and processing can affect the DNA concentration in plasma (5, 7, 8), but these findings do not explain the difference between plasma and serum concentrations of DNA mentioned above. Comparative investigations of the preanalytical conditions influencing the DNA concentration in serum and plasma are lacking. In addition, reference intervals for the concentration of cell-free DNA in serum were established without considering these factors (9). Thus, to complement the data of Lui et al. (5), we analyzed the influence of time delay in blood processing for plasma and serum at room temperature and at 4 °C.

Venous blood samples from 10 healthy volunteers (5 females and 5 males; mean age, 42 years) were simultaneously collected in Monovette plastic tubes without any additive for native serum (cat. no. 02.1726.001; Sarstedt), in plastic tubes with kaolin-coated plastic granulate coagulation accelerator (cat. no. 04.1904.01; Sarstedt) for preparation of serum samples, and in Monovette plastic tubes coated with potassium EDTA (cat. no. 05.1167.001; Sarstedt) for preparation of plasma samples. The tubes were either centrifuged at 2000g for 10 min at 4 °C immediately after venipuncture or were stored for 2, 4, or 8 h at room temperature (25 °C) and at 4 °C for 8 and 24 h, respectively, before being centrifuged under similar conditions. The supernatants were carefully removed and centrifuged again at 16 000g for 10 min at 4 °C and stored at −80 °C until analysis.

Using a NucleoSpin Blood Kit (Macherey-Nagel), we extracted DNA from 400 μL of sample per column, eluted it in 100 μL of buffer according to the manufacturer’s instructions, and stored the eluted DNA at −20 °C until use. DNA equivalents were quantified by amplifying the β-globin gene by real-time PCR (LightCycler™; Roche), using the QuantiTect SYBR Green PCR Kit (Qiagen). A 110-bp fragment of the β-globin gene was generated with the forward primer 5′- ACA-CAACTGTGTTCACTAGC-3′ and the reverse primer 5′-CAACT-TCATCCAGGTTCACC-3′ (TIB MolBiol) in a final concentration of 0.5 μM each. The cycle conditions were as follows: initial activation step at 95 °C for 15 min, followed by 45 cycles of denaturation at 95 °C for 15 s, annealing at 56 °C for 20 s, and extension at 72 °C for 15 s. The reaction volume was 20 μL, including 2 μL of DNA eluate. The product-specific melting temperature was ~82 °C. A calibration curve obtained with serial dilutions of a control DNA template (LightCycler Control Kit DNA; Roche) was linear at least up to 4546 genome-equivalents of β-globin [genome-equivalents/mL] were calculated using a conversion factor of 6.6 pg of DNA per single diploid human cell (5)].

The within-run (n = 12) and between-run (n = 10) imprecisions (CVs) for a DNA template (807.6 genome-equivalents/mL) were 3.1% and 7.2%, respectively. Statistical analyses were made with GraphPad Prism 3.03 for Windows (GraphPad Software). Parametric statistical tests were used because the DNA concentrations in our 10 samples had a gaussian distribution (Kolmogorov–Smirnov test).

There was no difference in the mean DNA concentrations (P = 0.729, paired t-test) in paired serum samples prepared with or without coagulation accelerator. We therefore compared the DNA concentrations in EDTA plasma with those in serum samples obtained in tubes without clot activation additive (Fig. 1). The DNA concentration in plasma did not change when blood samples were stored at room temperature for 8 h or at 4 °C for 24 h before being processed (Fig. 1). These results correspond to observations of Lui et al. (5), who analyzed the DNA concen-

![Fig. 1. DNA concentrations in plasma (left) and serum (right) aliquots prepared from blood samples stored at room temperature or at 4 °C and processed at different time intervals after phlebotomy. Data are mean (SE; error bars) β-globin concentrations (genome-equivalents/mL) determined by real-time quantitative PCR in samples of each from the 10 healthy adult volunteers. The intervals between blood collection and centrifugation of samples after storage at room temperature or at 4 °C (□) are indicated on the x axis. a and b indicate the significances, calculated by the Dunnett multiple-comparison test after parametric ANOVA analysis with repeated measures: a, at least P < 0.05 vs DNA in plasma samples processed immediately after collection; b, at least P < 0.05 vs DNA in serum samples processed immediately after collection.](image-url)
trations during a 6-h interval after phlebotomy. Our data (Fig. 1) confirm the observation that the DNA concentration is higher in serum than in plasma, as also shown by others (3–6).

The time delay and the storage temperature of blood before centrifugation had a significant impact on the DNA concentration in serum (Fig. 1). Compared with the DNA concentrations in plasma samples prepared immediately after venipuncture (0 h at room temperature), the DNA concentrations in serum samples were 2.3-fold higher when blood was stored for 0 h at room temperature and 8 h at 4 °C before centrifugation. The DNA concentration in serum increased ~3.8- to 4.8-fold when blood was stored for 2–8 h at room temperature and ~3-fold when blood was stored at 24 h at 4 °C compared with plasma values (0 h at room temperature).

DNA in serum rather than plasma has been reported to provide improved sensitivity for the detection of liver metastases in patients with colorectal carcinoma (4). This observation and our data indicate that the quantification of free circulating DNA in serum can be of diagnostic value only if the effect of time delay between blood collection and serum preparation is taken into account. When the serum DNA concentration is used as a potential marker for clinical disorders, strict standardization of serum preparation is mandatory. Reference intervals published recently obviously did not take this into consideration (9).

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**References**


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**Automated Enzymatic Assay for Homocysteine**

To the Editor:

Various methods have been developed for plasma total homocysteine (tHcy) measurement, including a tHcy enzyme conversion immunoassay designed for the Abbott IMx analyzer (1), a microtiter plate tHcy enzymatic immunoassay assay (2), HPLC methods (3–5), and gas chromatography–mass spectrometry methods (6). We have described a single-enzyme tHcy assay (enzymatic tHcy assay) based on a highly specific re-

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