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).

This work was supported in part by grants from the SÖNNENFELD-Stiftung Berlin. The study contains results of the doctoral thesis of M.L. This letter at http://www.clinchem.org/content/vol49/issue6/). Because the assay is based on an increase in absorbance over baseline, no blank without enzyme was used. The limit of quantification of the assay is 1.54 μmol/L, as determined by serial dilution of a pooled plasma calibrator (Sero logicals Corp.). Ten replicates of each dilution were analyzed. The limit of quantification is defined as the lowest concentration measured with a CV <20%. The assay is linear to at least 80 μmol/L tHcy, as determined visually after measurement of various amounts of homocysteine in plasma-buffered saline.

The within-run imprecision (CV) was 4.8% at 8.9 μmol/L tHcy, 3.0% at 14.9 μmol/L tHcy, and 4.5% at 25 μmol/L tHcy (n = 8). The between-assay CV over 10 days was 7.8%, 5.9%, and 4.9% at 8.8, 15, and 25 μmol/L, respectively. These imprecis-
sions are within ranges reported for currently used assays, including the Food and Drug Administration-cleared Bio-Rad HPLC assay (9, 10).

We assayed 121 plasma samples with the tHCY enzymatic assay on the Hitachi 912 (y) and with the Bio-Rad HPLC tHCY assay (x) (9, 10). The regression equation was: $y = 0.98x + 1.90$ μmol/L ($r = 0.977$; Fig. 1A). The mean (SD) difference between the tHCY enzymatic assay and the Bio-Rad HPLC tHCY assay (11–13) was $-1.62$ (2.33) μmol/L (Fig. 1B). Differences were not significantly correlated with homocysteine concentration (Pearson $r = 0.12$; $P = 0.185$).

Interference from l-CYS (0–200 μmol/L) was <10% at physiologic tHCY concentrations (0–200 μmol/L), and L-MET (0–200 μmol/L) showed no interference at these same tHCY concentrations.

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References
