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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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 recombinant form of L-homocysteine α,γ-lyase (rHCYase) *(7, 8)*. We report here the application of this tHCY enzymatic assay on the Hitachi 912 automatic chemistry analyzer. The principle of the assay is that rHCYase produces H_2S from tHCY and that the H_2S is quantified by its reaction with *N,N*-dibutylphenylenediamine, which produces a chromophore.

The assay uses four reagents and thus is compatible with implementation on the Hitachi 912. Briefly, 30 μL of EDTA plasma was incubated in a dithiothreitol reduction reaction (1 mmol/L dithiothreitol and 2 mL/L Triton X-100 in 40 mmol/L sodium phosphate buffer, pH 8.3) for 1.5 min to release bound homocysteine. The rHCYase reaction (0.05 g/L in 40 mmol/L sodium phosphate buffer, pH 8.3, containing 20 μmol/L pyridoxal 5-phosphate) was then run for 3.5 min. The *N,N*-dibutylphenylenediamine (12.5 mmol/L in 0.75 mol/L H_2SO_4) was then added, and 5 min later, an oxidant, 5 mmol/L $K_3Fe(CN)_6$ (in 10 mmol/L sodium phosphate buffer, pH 7.6), was added. Five minutes after addition of the oxidant, the end-points were read at wavelengths of 700 and 660 nm. (Assay conditions are given in detail in the Data Supplement accompanying the online version of 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 (Serologicals 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 phosphate-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 impreci-

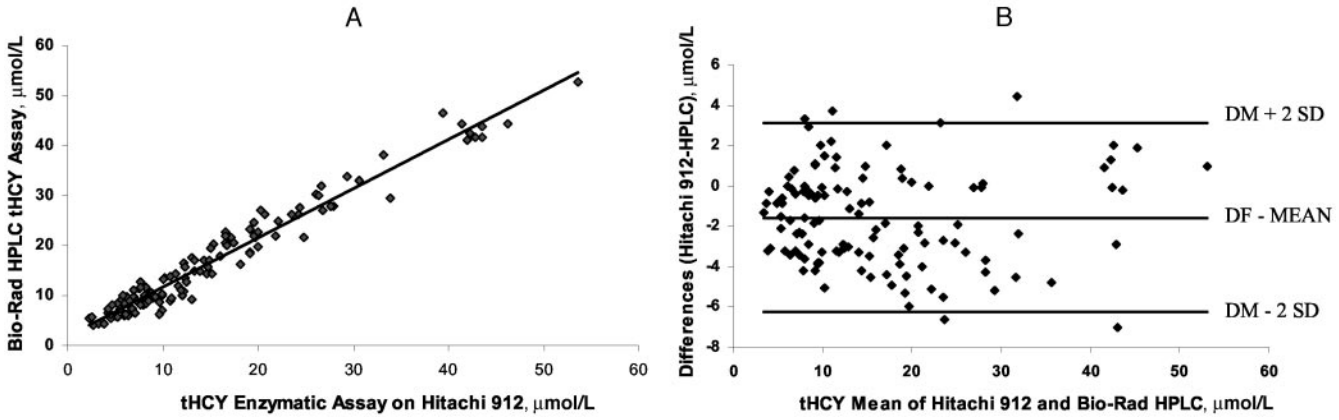

Fig. 1. Results for 121 samples measured by both the tHCY enzymatic assay on the Hitachi 912 and the Bio-Rad HPLC assay.

(*A*), linear regression analysis yielded the following equation: $y = 0.98x + 1.90$ μmol/L ($r = 0.977$). (*B*), Bland–Altman plot. The mean (SD) difference in tHCY was −1.6 (2.33) μmol/L. *DF*, difference; *DM*, mean difference.

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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