Evaluation of the Drew Scientific DS30 Homocysteine Assay in Comparison with the Centers for Disease Control and Prevention Reference HPLC Method, Ming Zhang, Elaine W. Gunter, and Christine M. Pfeiffer (National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA 30341; * author for correspondence; fax 770-488-4609, e-mail cpfeiffer@cdc.gov)

Determination of total homocysteine (tHcy) in plasma is becoming an important diagnostic procedure in clinical chemistry because a slightly increased concentration of tHcy in plasma has been discussed as an important independent risk factor for atherosclerotic diseases (1). Many methods, mostly by HPLC, have been reported for measuring tHcy (2). However, these methods are relatively complex and require highly specialized equipment. Drew Scientific, Inc. (company named for identification purposes only; this evaluation does not constitute an endorsement by CDC) has developed the DS30 tHcy system for measuring tHcy in plasma. We evaluated this new system and compared it with our CDC reference HPLC method (3).

The DS30 tHcy system comprises a small HPLC system using a 5-cm reversed-phase column, a tHcy assay reagent set that contains the necessary reagents and calibrators [5 and 20 \( \mu \text{mol/L} \) homocysteine (concentration equivalent to the free thiol)] and a quality-control (QC) set containing two concentrations of tHcy. The sample preparation requires 200 \( \mu \text{L} \) of plasma. A batch of 30 samples can be processed within 90 min. After the addition of 10 \( \mu \text{L} \) of the internal standard (IS; 2-mercaptoethylamine) to 200 \( \mu \text{L} \) plasma, the mixture of disulfides, mixed disulfides, and protein-bound thiols is reduced using 20 \( \mu \text{L} \) of tris(2-carboxyethyl)phosphine (TCEP). Protein is precipitated from this solution with trichloroacetic acid, and 100 \( \mu \text{L} \) of the supernatant is then derivatized with a fluorescent thiol-specific dye [ammonium 7-fluorobenzo-2-oxa-200-0-naphthalene (FBA)] in EDTA/borate buffer at 60 °C for 50 min. The thiol derivatives are separated in a subsequent step by HPLC and detected by their fluoresence. The total run time for a batch of 30 samples is ~5–6 h. Quantitative analysis is achieved using a two-point calibration curve with homocysteine in an aqueous matrix.

We assessed the within-assay and between-assay imprecision, the recovery of homocysteine added to plasma, the linearity of increasing tHcy concentration in plasma, the dilution linearity, and the limit of detection. We also compared the results of 260 plasma samples obtained by the DS30 system to the results we obtained previously for these samples by our CDC in-house HPLC method. These samples were a subset of the EDTA plasma samples for the National Health and Nutrition Examination Survey 1999+, which includes an omnibus informed consent and Human Subjects Review protocol.

The within-assay imprecision (CV) for five replicate measurements was <2% for the two concentrations of DS30 QC samples and the three concentrations of CDC QC pools. The between-assay imprecision was as follows: 5.8% for DS30 QC low (n = 21 days; mean tHcy, 13.4 \( \mu \text{mol/L} \)), 4.6% for DS30 QC high (n = 21 days; mean tHcy, 23.3 \( \mu \text{mol/L} \)), 3.8% for CDC QC low (n = 27 days; mean tHcy, 7.3 \( \mu \text{mol/L} \)), 3.2% for CDC QC medium (n = 27 days; mean tHcy, 14.7 \( \mu \text{mol/L} \)), and 4.8% for CDC QC high (n = 16 days; mean, tHcy, 31.8 \( \mu \text{mol/L} \)).

Homocysteine was added to a plasma sample at six different concentrations in duplicate: 0, 6.25, 12.5, 25, 50, and 100 \( \mu \text{mol/L} \) (concentrations equivalent to the free thiol). The mean recovery (SD) was 101.7% ± 1%. The linearity of increasing tHcy concentrations in plasma was very good up to 100 \( \mu \text{mol/L} \) \((y = 1.028x – 0.127; r^2 = 1.000)\).

The DS30 HPLC system is programmed such that peak recognition of tHcy and the IS depends on a minimum concentration of cysteine and cysteinyl-glycine. We found that this minimum concentration was 50 \( \mu \text{mol/L} \) cysteine and 7.5 \( \mu \text{mol/L} \) cysteinyl-glycine. Thus, dilution of plasma >1:4 with saline or water leads to a loss of recognition of the peaks. Dilution of a plasma sample (tHcy concentration of ~30 \( \mu \text{mol/L} \)) with a solution containing 200 \( \mu \text{mol/L} \) cysteine and 30 \( \mu \text{mol/L} \) cysteinyl-glycine [these concentrations of cysteine and cysteinyl-glycine correspond to an average concentration found in the population (4) at 1:2, 1:4, 1:8, and 1:16 gave very good linearity \((y = 0.999x + 0.115; r^2 = 0.999)\). The difference between the measured and the expected concentrations was <3%. Diluting the same plasma with either saline or water at 1:2 and 1:4 also gave very good linearity; however, the difference between the measured and the expected concentrations was 5–10%. The limit of detection for tHcy was ~2 \( \mu \text{mol/L} \).

The retention times for each compound were stable over the 24 assays we performed over 4 months. The variation of the retention times for all compounds was 3%. The variation (CV) of the IS heights for all samples, except for the DS30 QC samples, over the period of evaluation was 10.5%. The DS30 QC samples gave consistently lower heights (~25% lower) for the IS than all other samples. The reason for this was that the DS30 QC samples were not EDTA plasma, but heparin plasma, and apparently the anticoagulant influenced the obtained peak height. However, because ratios between tHcy and the IS heights were used for quantification, this should not have influenced the final result. However, users should be alerted that we tested only the suitability of the DS30 assay for EDTA plasma samples. We believe the use of this assay for matrices other than EDTA plasma should be evaluated first.

An extensive method comparison was performed between the DS30 tHcy system and the CDC in-house HPLC method. If samples were not analyzed simultaneously with both methods, they were stored at ~70 °C for not >6 months between the assays. The performance of the CDC reference HPLC assay and the validation results were described in detail in a separate article (3). tHcy concentrations determined for 260 plasma samples from healthy subjects gave good correlation between the two methods: \(y = 1.018x + 0.280\) \((r^2 = 0.976)\). The Bland–Altman plot in
The classification of pleural effusions as transudates or exudates often is an early step in their evaluation (1–4). Exudates but not transudates require additional and often invasive diagnostic procedures. The criteria of Light et al. (5) for classification of pleural effusions have been reported to lead to unwarranted invasive diagnostic procedures in 20–30% of patients with a transudate (1, 3) and to misclassify some exudates as transudates (3, 4).

Protein analysis has been proposed to improve classification into transudates or exudates based on the assumption that large proteins are present only in exudates because of the increased capillary permeability (1). Agarose gel electrophoresis (AGE) has been the standard procedure for serum protein fractionation for >30 years, but it is laborious and methods do not agree (6–8).

Capillary zone electrophoresis (CZE) is an attractive alternative (6, 9, 10) and avoids between-protein differences in dye affinity.

We evaluated the Beckman Paragon CZE™ 2000 system, a multicapillary instrument for automated serum protein electrophoresis (7, 8), for quantitative fractionation of proteins in body fluids.

For method comparison, leftover samples from 49 pleural and 11 ascitic fluids from 47 inpatients were used. There were no preset selection criteria regarding age, sex, or type of disease (11); however, only 37 pleural effusions were classified as transudates or exudates after exclusion of patients lacking definitive clinical diagnosis (n = 5), patients with multiple diagnoses (n = 3), or in case of interference by radioopaque agents (n = 4). A serum sample was collected simultaneously with the pleural effusion.

This study was supported by Drew Scientific, Inc., who provided the DS30 instrument as a loaner and all the reagents and columns needed to perform this evaluation. An abstract containing the summary of this evaluation has been submitted to Experimental Biology 2001 (published in FASEB J 2001;15:A613).