Fig. 1 is shown with the bias between the two methods by plotting for each sample the difference of results from the two methods (y axis) compared with the mean of results (x axis). The mean difference for the DS30 showed a slight positive bias of 0.39 μmol/L (95% confidence interval, 0.30–0.49). The central 0.95 interval (mean difference, ±2 SD) indicates the agreement between the two methods. Ninety-five percent of tHcy determinations by DS30 were 1.10–1.89 μmol/L higher than concentrations determined by the CDC HPLC method.

In conclusion, the DS30 tHcy system showed within-assay and between-assay imprecision (CV <6%) comparable to other frequently used HPLC assays (3–5) and the Abbott IMx assay (6). The DS30 tHcy system also showed complete recovery of added tHcy and a linearity up to 100 μmol/L. Dilution of plasma with a solution containing cysteine and cysteinyl-glycine produced very good linearity. However, dilutions of plasma with water or saline should not exceed 1:4 to avoid misidentification of peaks resulting from undetectable cysteine and cysteinyl-glycine peak heights. Samples with a tHcy concentration up to maximum 400 μmol/L can be measured after 1:4 dilution with water or saline. However, we recommend verifying the printout with regard to peak identification, retention times, and peak heights to avoid mislabeling peaks (especially with diluted samples). The comparison of this method with the CDC reference HPLC method on samples up to 25 μmol/L tHcy revealed only a minimal bias (0.39 μmol/L). Therefore, the DS30 tHcy system performed accurately and precisely, and thus might be well suited for routine measurement for tHcy where complex HPLC analysis is not feasible.

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).

**References**


**Capillary Zone Electrophoresis of Proteins in Body Fluids: Comparison of Capillary and Agarose Gel Electrophoresis**, Roxane Claey, Chris Groven, and Frans K. Gorus (Department of Clinical Chemistry, Academic Hospital Vrije Universiteit Brussel (AZ-VUB), Laarbeeklaan 101, B-1090 Brussels, Belgium; *author for correspondence: fax 32-2-477-5047, e-mail lchmgsf@az.vub.ac.be)

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
fluid (n = 30) or within 24 h before (n = 13) or after (n = 17) the puncture. Final diagnosis was retrieved from the patient’s file based on the conclusions reached by physicians from the Department of Internal Medicine, who were unaware of the results of the method comparison, and after review of all available clinical, anatomicopathological, and microbiological reports.

Vitros™ slides (Ortho Clinical Diagnostics) were used for total protein and lactate dehydrogenase (LD) measurement. Albumin in fluids was measured immunonephelometrically on a BNA™ analyzer (Dade Behring).

AGE was performed on the REP™ system (Helena Laboratories) using Ponceau S staining. CZE was carried out with Paragon CZE 2000. When necessary for CZE, fluids were concentrated (Centricon filters; cutoff, M_r 30 000; Millipore Corporation) to total protein concentrations ≥35 g/L. All electrophoretograms were independently interpreted by two laboratory investigators blinded for the final clinical diagnosis. The study was performed in accordance with the current revision of the Helsinki Declaration of 1975 (12).

The selected 37 pleural fluids (see above) were classified as exudates (n = 26) or transudates (n = 11) according to the patient’s clinical diagnosis [for criteria, see Ref. (1)] and compared with their classification (a) according to Light et al. (5) when at least one of the following criteria was met: (i) pleural fluid-serum total protein ratio >0.5, (ii) pleural fluid-serum LD ratio >0.6, and/or (iii) pleural fluid LD more than two-thirds of the upper reference limit of serum LD; or (b) according to Chen and Lam (1) when any of the α_2-globulin, β-lipoprotein, and γ-globulin bands were detected in the electrophoretogram (serum-like pattern on AGE).

Paired Wilcoxon tests and unpaired Mann–Whitney U-tests were performed two-tailed with Analyze-It for Microsoft Excel (significance threshold <0.05). Linear or Passing-Bablok regression analysis was used for method comparisons. ROC curve analysis was performed with MedCalc.

Forty-nine pleural and 11 ascitic fluids were analyzed with CZE and AGE. In four pleural and one ascitic fluid samples, excluded from statistical analysis, radioopaque agents caused a spurious peak in the α_2-globulin fraction in the CZE electrophoretograms, as has been observed in serum (13).

The median total protein concentration (range) was 32.0 g/L (1.0–65.0 g/L) for the remaining 45 pleural fluids and 17.0 g/L (9.0–30.0 g/L) for the 10 ascitic fluids. Results obtained by CZE and AGE were statistically different for albumin and α_1-, α_2-, β-, and γ-globulins (P ≤0.001) but were very significantly correlated (see Table 1). Albumin concentrations determined electrophoretically correlated significantly with nephelometric data (Table 1); values obtained by AGE, but not by CZE, were higher than those obtained by the BNA method (P <0.0001). As in serum, values obtained by CZE for the α_1- and β-globulin fractions were 30% and 15% higher, respectively, because of detection of α_1-acid glycoprotein and C_r complement, which were not detected by AGE (7, 8, 10). In 20 samples, a pseudomononal fibrinogen peak was detected in the γ-globulin fraction by AGE, but not by CZE. γ-Globulin fractions measured by AGE were higher in samples with (P <0.006) and without fibrinogen (P = 0.03; n = 35). In one pleural effusion, a monoclonal (IgG λ) peak was detected in the γ-globulin fraction in the AGE (11.7 g/L) and CZE (12.0 g/L) electrophoretograms.

Thirty-seven pleural fluids were clinically classified as exudates (n = 26; 10 patients with malignancies, 13 with pneumonic and parapneumonic effusions, 3 with Dressler syndrome) or as transudates [n = 11; 10 patients with congestive heart failure and 1 with (pseudo)Meigs syndrome].

Electrophoretic patterns of unconcentrated pleural effusions are shown in Fig. 1. By visual inspection of AGE electrophoretograms, all exudates (n = 26) but only 7 of 11 transudates were correctly classified vs 24 of 26 exudates and 10 of 11 transudates correctly classified with the criteria of Light et al. (5). Consistent with other data (3, 4), the criteria of Light et al. (5) achieved 92% diagnostic sensitivity (95% confidence interval (CI), 75–99%) and 91% specificity (95% CI, 59–100%) for exudates and correctly classified 92% of the fluids. Visual interpretation of AGE electrophoretograms achieved 100% sensitivity (95% CI, 87–100%) and 64% specificity (95% CI, 31–89%) with an overall 89% diagnostic efficiency, consistent with Chen and Lam (1). Visual inspection of AGE electrophoretograms complemented the criteria of Light et al. (5) in diagnosing two otherwise undetected malignant exudative effusions; conversely, the criteria of Light et al. identified three transudates attributable to congestive heart failure [valvular dysfunction (n = 2) and cor pulmonale (n = 1)] in addition to those detected by visual inspection of AGE electrophoretograms. The pleural effu-

### Table 1. Linear regression and Passing-Bablok regression equations for 55 body fluids.

<table>
<thead>
<tr>
<th>Protein fraction</th>
<th>Linear regression</th>
<th>S_daf g/L</th>
<th>r</th>
<th>Passing-Bablok</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>CZE = 0.91 (AGE) - 0.05</td>
<td>1.1</td>
<td>0.99</td>
<td>CZE = 0.91 (AGE) - 0.10</td>
</tr>
<tr>
<td>CZE vs AGE</td>
<td>CZE = 1.08 (BNA) - 0.06</td>
<td>0.1</td>
<td>0.99</td>
<td>CZE = 1.09 (BNA) - 0.07</td>
</tr>
<tr>
<td>CZE vs BNA (n = 39)</td>
<td>AGE = 1.16 (BNA) + 0.39</td>
<td>0.2</td>
<td>0.98</td>
<td>AGE = 1.19 (BNA) + 0.003</td>
</tr>
<tr>
<td>α_1-Globulins</td>
<td>CZE = 1.29 (AGE) + 0.40</td>
<td>0.4</td>
<td>0.96</td>
<td>CZE = 1.33 (AGE) + 0.27</td>
</tr>
<tr>
<td>α_2-Globulins</td>
<td>CZE = 1.04 (AGE) + 0.54</td>
<td>0.5</td>
<td>0.96</td>
<td>CZE = 1.26 (AGE) + 0.12</td>
</tr>
<tr>
<td>β-Globulins</td>
<td>CZE = 1.15 (AGE) + 0.43</td>
<td>0.6</td>
<td>0.99</td>
<td>CZE = 1.22 (AGE) - 0.18</td>
</tr>
<tr>
<td>γ-Globulins</td>
<td>CZE = 0.89 (AGE) + 0.14</td>
<td>0.7</td>
<td>0.97</td>
<td>CZE = 0.94 (AGE) + 0.06</td>
</tr>
</tbody>
</table>
sion caused by the Meigs syndrome was assigned as exudate by both criteria. Qualitative evaluation of CZE electrophoretograms was not performed because concentration steps before analysis (see above) could visualize small $\alpha_2$- and $\gamma$-globulin bands in transudates, whereas $\beta$-lipoprotein bands are not observed in CZE 2000 electrophoretograms (10). Quantitative analysis was performed on 26 exudates and 11 transudates, applied at similar concentrations in both methods. Electrophoretically fractionated (not shown) and total protein concentrations were significantly higher ($P < 0.05$) in exudates [median (range), 35.0 g/L (11.0–65.0 g/L) vs 18.0 g/L (1.0–31.0 g/L) for transudates]. The $\alpha_2$-albumin ratio was lower ($P < 0.001$) in transudates [median ratio (range), 0.10 (0.09–0.22) vs 0.19 (0.06–0.59) for exudates with CZE; 0.06 (0.05–0.11) vs 0.12 (0.05–0.40) for exudates with AGE]. Cutoff values for optimal diagnostic efficiency determined by ROC curve analysis (Fig. 1) were $>0.14$ for CZE and $>0.11$ for AGE; the areas under the ROC curves were 0.85 (95% CI, 0.70–0.95) and 0.83 (95% CI, 0.68–0.94), respectively. The established cutoff values for $\alpha_2$:albumin ratios are lower than those calculated (using Beckman SPE gels) by Chen and Lam (1), who classified effusions by electrophoretic instead of clinical criteria. Clinical classification, however, is limited by the fact that some pleural effusions are mixtures of transudates and exudates (1).

For CZE the established cutoff value for the $\alpha_2$:albumin ratio achieved a diagnostic sensitivity and specificity for exudates of 81% (95% CI, 61–93) and 91% (95% CI, 59–99), respectively, with 84% correctly classified fluids. Likewise, AGE achieved a diagnostic sensitivity of 62% (95% CI, 44–80) and specificity of 100% (95% CI, 100–100) with 73% efficiency, which is not significantly different from all other criteria used because of overlapping CIs. Nevertheless, compliance with the criteria of Light et al. (5) for exudates or with CZE $\alpha_2$:albumin ratio $>0.14$ correctly classified all exudates, but with a specificity of 82% (two transudates still being classified as exudates). With an AGE $\alpha_2$:albumin ratio $>0.11$ as criterion, two malignant effusions were still erroneously diagnosed as transudates. Thus, combined use of the criteria of Light et al. and CZE quantification can achieve 100% sensitivity for exudates, but may still lead to unwarranted investigations in a

Fig. 1. Electrophoretic patterns obtained by REP AGE on unconcentrated pleural transudates and exudates (A) and plots of ROC curve analysis for detection of the optimal cutoff of the $\alpha_2$:albumin ratio for AGE (B) and CZE (C). (A), the reference sample (REF) is a pooled human serum sample (stored in aliquots at $-80^\circ$ C). The effusions (P) are paired with the accompanying serum samples (S). The first three effusions, P1, P2, and P3, represent typical pleural transudates and have total protein concentrations of 18, 18, and 13 g/L, respectively; only the albumin and transferrin bands are visible. P4 and P5 are two exudative effusions with total protein concentrations of 64 and 27 g/L, respectively. In P4, $\alpha_2$-globulin, $\beta$-lipoprotein, and $\gamma$-globulin bands are present; in P5, a prominent $\alpha_2$ and a $\gamma$-globulin band are present, but the $\beta$-lipoprotein bands are barely visible. (B and C), the dotted line represents the line of no discrimination. ● indicates the cutoff value for optimal diagnostic efficiency in each panel.
limited number of patients because of their suboptimal specificity.

In conclusion, the Beckman Paragon CZE 2000 analyzer can be used for the fractionation of proteins in pleural and ascitic fluids. The results correlate significantly with those obtained by REP AGE. Albumin concentrations measured by CZE agree better with immunonephelometrically determined values on the BNA analyzer than do results obtained by AGE. Protein zone electrophoresis could complement the criteria of Light et al. \[\text{5}\] by increasing the sensitivity of the detection of exudative pleural effusions. The cutoff for the $\alpha_2: \text{albumin ratio on CZE achieved a slightly, albeit not significantly, higher sensitivity for exudates than the same ratio on REP.}\n
We gratefully acknowledge the invaluable technical assistance of V. Baeten and S. Exterbille.

References