Can Heparin Plasma Be Used instead of Serum for Nephelometric Analysis of Serum Proteins?

To the Editor:

Nephelometry is widely used for quantification of serum proteins. The main manufacturers of nephelometers, Beckman-Coulter and Dade-Behring, recommend serum as a sample for most tests. For a limited number of tests, e.g., lipoprotein(a), they state that serum and heparin plasma can be used. Serum should be separated from contact with cells as soon as possible (1), but blood should be allowed to sit long enough for complete clot formation because inadequate clot formation with residual fibrin strands can clog the sampling pipette. Use of plasma circumvents these clotting-related problems. We therefore investigated whether lithium-heparin plasma can be used for nephelometric analysis of a whole array of serum proteins.

Serum and plasma samples were collected simultaneously. The serum clot contact time was 4 h, whereas plasma was separated from cells within 2 h (1). Analysis was performed with an Immage instrument (Beckman-Coulter) for the following 22 analytes: albumin, prealbumin (PAB), α1-acid glycoprotein, α1-antitrypsin, α2-macroglobulin (AMG), β2-microglobulin, factor B, C1 inhibitor, C1q, C3, C4, C5, ceruloplasmin (CERU), IgA, IgG, IgGκ, IgGμ, IgGδ, IgGλ, IgM, and κ and λ light chains. All reagents were from Beckman-Coulter except for the reagents for IgG subclasses, which were from Sanguini, and the reagents for C1q, C5, C1 inhibitor, and β2-microglobulin, which were supplied by DakoCytomation. Samples were analyzed daily for albumin, PAB, α1-antitrypsin, C3, C4, C5, CERU, IgA, IgG, and IgM. The other assays were performed weekly with preanalytical storage of samples at 4 °C for a maximum of 6 days. The samples included normal, low, and/or increased values.

For the sample comparison, Pearson correlation (Analyze-It for Microsoft Excel, Ver. 1.62), Passing–Bablok, and Bland–Altman analyses were performed (2). For the evaluation of clinical significance, the reference-change value (RCV) was calculated (3) with Z being the 0.975 percentile of the Gaussian distribution, and CVa and CVb being the analytical and intra-individual CVs of the test, respectively:

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\sqrt{2} \times Z \times \sqrt{CV_a^2 + CV_b^2}
\]

The multiplication by \(\sqrt{2}\) is because 2 values are being compared. The intra-individual CV was obtained from Westgard QC (4), and the analytical CV was estimated by a 4-month cumulative CV of quality-control results at an analyte concentration close to the sample value.

In total, 508 analyses were performed on 170 serum and 170 plasma samples from 163 patients (84 males and 79 females). The Pearson correlation revealed excellent correlation, except for IgGκ, which had an outlier. Omitting the outlier gave better correlation (correlation coefficient = 0.99; 95% confidence interval, 0.98–1.0; Table 1). Passing–Bablok and Bland–Altman analyses revealed no significant differences (\(P < 0.05\)) between plasma and serum for 15 and 12 of the 22 tests, respectively. Eleven and 3 tests (C4, CERU, and λ light chain) were significantly different in either or both statistical analyses, respectively (Table 1).

We next evaluated whether the statistically significant differences observed for these 14 tests were also clinically relevant by the RCV technique. This analysis revealed no clinically relevant differences between plasma and serum values, except for 1 AMG determination (of 24).

Passing–Bablok analysis revealed (a) a constant bias for AMG, (b) a proportional bias for IgA, and (c) a nonlinear relationship between serum and heparin plasma for PAB and IgGκ. Visual examination of the regression line for PAB and IgGκ showed only slight nonlinearity. We further evaluated the nonlinearity for these 2 tests by use of the cusum test, as described in detail by Passing and Bablok (5). Briefly, a score is assigned to each data point: 0 for data points on the regression line and \(\pm \sqrt{\text{RTT}(L/I)}\) and \(\pm \sqrt{\text{RTT}(I/L)}\) for measurements above and under the regression line, respectively (L denotes the number of measurements under the line and I those above). The data points are sorted along the fitted line, and cumulative sums of sorted scores are computed. If any of these cusums exceeds the threshold for a given significance level, nonlinearity can be concluded. For both PAB and IgGκ, the threshold was exceeded at the 5% significance level (\(P < 0.05\)), but not at the 1% significance level (\(P > 0.01\)).


In conclusion, our results suggest that for nephelometric quantification of specific proteins, serum and lithium-heparin plasma samples can be used interchangeably. It should be mentioned that plasma samples may form a cryoprecipitate when stored. This can be resolved with additional centrifugation (6).

References


Mieke Develter¹
Norbert Blanckaert¹
Arnout Komárek²
Xavier Bossuyt³

¹ Laboratory Medicine
Immunology
University Hospital Leuven
Leuven, Belgium

² Department of Biostatistics
University Leuven
Leuven, Belgium

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