hemolysis in patients exhibiting a concurrent acute-phase reaction as indicated by increases in C-reactive protein. The sensitivity of haptoglobin at a cutoff of 250 mg/L, however, is somewhat less than in situations where only hemolysis is present (2). Nonetheless, it is apparent that the decline in serum haptoglobin concentrations, owing to its complexing with hemoglobin and eventual removal via the reticuloendothelial system, occurs significantly faster than serum haptoglobin increases as an acute-phase reactant.

References

Value of Polyacrylamide Gradient Gel Electrophoresis of Lipoproteins for Determining HDL Cholesterol, F. Collais (Laboratoire d’Hygiène, de Prévention et d’Education Sanitaire, Université René Descartes, 4 avenue de l’Observatoire, 75270 Paris Cedex 06, France), D. Roche (Laboratoire de Biochimie, Hôpital Laennec, Paris), and J. P. Andreux (Laboratoire d’Hématologie, Hôpital Charles Foix, Ivry sur Seine)

Given the great clinical importance of high-density lipoprotein cholesterol (HDLc), the development of a simple, routine method for its accurate quantification is in urgent demand. The reference method, ultracentrifugation, is expensive, laborious, and time-consuming. On the other hand, the more frequently used selective precipitation of lipoproteins under- or overestimates HDLC and remains inappropriate for hypertriglyceridemic samples. Polyacrylamide gradient gel electrophoresis (PAGE) with pre-staining by Sudan Black B, currently used for classifying various lipoproteinemia, can also be used for densitometric determination of HDLC. HDLC (mmol/L) = HDL (%) × total cholesterol (mmol/L).

This application of PAGE has been described by others (1, 2), but questions of its specificity have generated much controversy. The effect of overloading with purified fractions of high-, low-, and very-low-density lipoproteins has been previously reported (2), so we evaluated the quantification of cholesterol in the gel matrix and compared the results with those obtained by PAGE densitometry. We assayed in triplicate 50 sera from fasting subjects (total cholesterol from 3.30 to 6.00 mmol/L; triglycerides from 0.40 to 1.60 mmol/L; phospholipids from 2.00 to 3.10 mmol/L), using ready-made cylindrical Lipophor® gels (Miles Laboratories, Elkhart, IN 46514). We used the reported conditions (1, 2), but with 0.200 mL of a "gel support" without staining, for electrophoretic separation. The "separation gel" was then removed and we cut out a 5-mm-wide zone of gel to isolate the HDL fraction.

To assay the cholesterol in this fraction, we placed each 5-mm segment of gel in a 2-mL glass syringe fitted with a 10/8 gauge needle. Exerting pressure on the piston, we could extrude a fairly fine gel suspension. We rinsed the syringe with 0.100 mL of a 0.15 mol/L NaCl solution and combined the rinse with the gel suspension. The method was calibrated with adding 20 µL of a 5.17 mmol/L cholesterol calibrator to the same volume of "separation gel" and treating as described. We found the calibrator was well taken up in the matrix gel after a 1-h contact. A reaction blank consisted of a segment of pure gel treated under the same conditions. In each fraction, we determined the cholesterol enzymatically by adding 1 mL of cholesterol assay reagent (Bio Mérieux, Marcy l’Etoile, 69260 France) to the extruded gel plus the rinsing solution. Before reading the absorbance at 510 nm against the blank, we centrifuged the tubes at 5000 rpm for 10 min to eliminate any gel debris. Regression data [y = densitometric determination of HDLC; x = evaluation in the gel matrix] were: y = 0.87x + 0.34 (r = 0.95).

We also determined whether such a HDLC quantification depended on the triglycerides concentration. We assayed 96 sera from normal subjects or patients with type IIA, IIB, or IV hypertriglyceridemia, divided into three groups with identical concentrations of total cholesterol (TC) and HDLC (mean ± SD, mmol/L): Group I: TC = 5.10 ± 0.070, HDLC = 1.50 ± 0.012; Group II: TC = 7.60 ± 1.00, HDLC = 1.50 ± 0.060; Group III: TC = 6.20 ± 0.100, HDLC = 1.20 ± 0.015. We then divided each group into three sub-groups with low (<0.50 mmol/L), normal (>0.92, <1.30), and high (>2.00, >2.70) triglyceride concentrations. Intragroup analysis of variance with F <2.77 and P nonsignificant (>0.05) demonstrated that densitometric evaluation of HDLC was independent of triglyceride concentrations.

References

Measurement of Apolipoprotein A-I in Serum with the Technicon RA-1000 System, Glenn R. Irish, Thomas B. Leduc, and Denny M. Barrantes (Atlantic Antibodies, 10 Nonesuch Rd., Scarborough, ME 04074)

Since 1979, apolipoproteins A-I and B and their ratio have been viewed as potent predictors of risk to coronary artery disease (1–3). This concept has led to the development of assays for the quantification of these apolipoproteins with analytical tools common in the clinical laboratory. We evaluated a commercially available kit for the turbidimetric assay of apolipoprotein A-I (Atlantic Antibodies, Scarborough, ME 04074) on the RA-1000 System (Technicon Instruments, Tarrytown, NY 10591), a random-access clinical chemistry analyzer. We found this assay to be precise, accurate, and rapid.

Assay-specific parameters were programmed according to the package insert accompanying this kit. Antibody reagent was diluted with the Apo A-I Antibody Diluent provided, which contains a detergent and does not require filtration. Calibration materials and test samples were diluted with the Saline Diluent provided. Microvolumes of antibody and calibration standards were pipetted into reaction cuvettes to calibrate the instrument. Subsequent to calibration, test samples were assayed and concentrations determined from the stored curve. Analysis of up to 20 samples was complete within 20 min.

Within-run and day-to-day imprecision at each of three
Immunoturbidimetry of Apolipoprotein B in Human Serum, Glenn R. Irish, Denny M. Barrantes, and Thomas B. Ledue (Atlantic Antibodies, 10 Nonesuch Rd., Scarborough, ME 04074)

Measurements of apolipoproteins A-I and B and their ratio have been suggested as better predictors of risk for coronary artery disease than either lipids or lipoproteins (1–3). Thus numerous and diverse assays have been developed for quantification of these apolipoproteins. Many of these assays are characterized by one or more substantive disadvantages to a clinician, such as poor precision or sensitivity; long, tedious assay preparation and long analysis time; rapid reagent deterioration; or need for expensive, dedicated instrumentation. We evaluated our commercially available kit (Atlantic Antibodies) for the turbidimetric assay of apolipoprotein B in the Cobas Bio (Roche Analytical Instruments, Nutley, NJ 07110) and found it to be exempt from these major performance drawbacks.

Cobas Bio operation and programming has been described previously (4). Assay-specific parameters were programmed according to the package insert accompanying this kit. Antibody reagent was diluted with the Polymer Diluent provided and does not require filtration. Calibration materials and test samples were diluted with the Saline Diluent provided. Microvolumes of antibody and sample (antigen) were simultaneously pipetted into reaction cuvettes. Analysis was complete within 20 min.

Within-run and day-to-day CVs at each of three apolipoprotein B concentrations (0.76, 1.45, and 2.20 g/L) were <4%. Analytical recovery averaged 99% in the range of 0.71 to 1.61 g/L. Comparison was made with a rate-nephelometric method (n = 98, r = 0.97, y = 0.101x + 0.04 g/L). Up to 5 g of added hemoglobin or 0.15 g of added bilirubin per liter did not interfere.

Calibration materials for this kit, provided in liquid form, remained homogeneous in appearance and consistent in apolipoprotein B concentration for at least eight weeks at 4 °C. These reagents were not affected by repeated freezing and thawing (10 cycles). Accelerated degradation studies predicted stability of longer than two years for calibration materials stored at -20 °C.

References

Do Biological-Variation Data Shed Light on the Demise of Some Historical Indices Used to Assess Calcium Homeostasis? Elizabeth M. S. Gowans and Callum G. Fraser (Dept. Biochem. Med., Ninewells Hosp. and Med. School, Dundee DD1 9SY, Scotland)

Although newer types of tests aid investigation of disorders of calcium homeostasis, we examined the biological variation of some current and historical indices (I), namely, serum calcium concentration [Ca] corrected for serum albumin concentration [Alb], urinary calcium/creatinine ratio, phosphate/creatinine clearance ratio (C), tubular reabsorption of phosphate [(1 – C) 100%], and phosphate excretion index [C – (0.17 serum phosphate concentration, mmol/L – 0.07)]. We corrected serum [Ca], mmol/L, by using our usual formula, namely, corrected [Ca] = found [Ca] + 0.023 (40 – serum [Alb], g/L).

The overall means, and average intra-individual (CV) and interindividual (CVG) variations were calculated with singlicate data as described previously (2, 3). Analytical variation (CVA) was derived by summation of component errors. The following tabulation shows the means and the components of variation (as CV, %) indices of individuality (4), calculated as CV/100; and the critical differences (CD) for serial results to be significantly (P <.05) different, expressed as percentages of the mean values.

<table>
<thead>
<tr>
<th></th>
<th>CV&lt;sub&gt;A&lt;/sub&gt;</th>
<th>CV&lt;sub&gt;I&lt;/sub&gt;</th>
<th>CV&lt;sub&gt;G&lt;/sub&gt;</th>
<th>CV&lt;sub&gt;G&lt;/sub&gt;/CV&lt;sub&gt;A&lt;/sub&gt;</th>
<th>CD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum calcium</td>
<td>2.41 mmol/L</td>
<td>1.2</td>
<td>2.1</td>
<td>1.5</td>
<td>1.39</td>
</tr>
<tr>
<td>Serum corrected calcium</td>
<td>2.30 mmol/L</td>
<td>1.7</td>
<td>1.2</td>
<td>1.6</td>
<td>0.77</td>
</tr>
<tr>
<td>Urinary calcium</td>
<td>3.52 mmol/L</td>
<td>3.0</td>
<td>26.2</td>
<td>24.3</td>
<td>3.13</td>
</tr>
<tr>
<td>Urinary calcium/creatinine ratio</td>
<td>0.37</td>
<td>0.7</td>
<td>25.6</td>
<td>30.5</td>
<td>0.84</td>
</tr>
<tr>
<td>Urinary phosphate</td>
<td>24.8 mmol/L</td>
<td>1.8</td>
<td>19.4</td>
<td>30.9</td>
<td>0.63</td>
</tr>
<tr>
<td>Phosphate/creatinine clearance ratio</td>
<td>0.16</td>
<td>4.1</td>
<td>18.9</td>
<td>17.8</td>
<td>1.12</td>
</tr>
<tr>
<td>Tubular reabsorption of phosphate</td>
<td>84.3%</td>
<td>0.7</td>
<td>2.7</td>
<td>3.3</td>
<td>1.06</td>
</tr>
<tr>
<td>Phosphate excretion index</td>
<td>0.034</td>
<td>4.2</td>
<td>111.6</td>
<td>105.3</td>
<td>1.06</td>
</tr>
</tbody>
</table>

When serum [Ca] is corrected for serum [Alb], the intra-individual variation and critical difference decline while, as