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References

Comparison of the Du Pont aca and Dow Methods for Determination of High-Density Lipoprotein Cholesterol

Nadja N. Rehak, Ronald J. Elin, Ruth Chesler, and Ernestine Johnson

We compared the Du Pont aca (phosphotungstate–enzymic cholesterol) and the Dow (dextran sulfate/Mg2+–enzymic cholesterol) methods for the determination of high-density lipoprotein cholesterol (HDLc) and total cholesterol in serum from 113 patients. The aca results for both total cholesterol and HDLc were significantly greater (p < 0.0001) than the Dow results, the aca method overestimating the HDLc concentration (mean recovery 107.2%) in serum samples with values assigned by the Centers for Disease Control. The precision of the aca method for HDLc was essentially the same as that of the Dow method. Bilirubin (up to 0.17 g/L), hemoglobin (up to 4 g/L), and slight lipemia (triglycerides up to 5.4 g/L) did not interfere with the aca method.

Additional Keyphrases: variation, source of enzymic methods

Recently, a semi-automated enzymic method for high-density lipoprotein cholesterol (HDLc)1 became available on the Du Pont Automatic Clinical Analyzer (aca). The HDLc is separated from other cholesterol by precipitation of apo B-lipoproteins with buffered phosphotungstate. We compared this method with a dextran sulfate/Mg2+–enzymic HDLC method and evaluated the method for interference by bilirubin, hemoglobin, and lipemia. In addition, we compared the Du Pont and the Dow methods for the determination of total cholesterol (TC) in serum.

Materials and Methods

We used an aca with computer II (Du Pont, aca Division, Wilmington, DE 19898) and a Model 250 spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, OH 44074).

Reagents

* * * *

Aqueous bilirubin, 0.5 g/L. Dissolve 12.5 mg of bilirubin (Standard Reference Material no. 916; National Bureau of Standards, Gaithersburg, MD) in 1 mL of dimethyl sulfoxide in a 25-mL volumetric flask. Add 2 mL of aqueous Na2CO3 (0.1 mol/L) and 18 mL of a 70 g/L solution of human serum albumin (Fraction V, cat. no. A2386; Sigma Chemical Co., St. Louis, MO 63178). Neutralize the solution (pH 7.0) by adding 2 mL of 0.1 mol/L HCl and dilute to volume with the human serum albumin solution. A bilirubin blank solution is prepared by the same procedure, but omitting the bilirubin.

Controls. To estimate precision, we used two control sera (Precisp, cat. no. 125067; Bio-Dynamics/bmc, Indianapolis, IN 46250) and Monitrol I, cat. no. 85103-1, Dade, Miami, FL 33152) and pooled human serum, prepared by the laboratory and stored at −20 °C.

Standards. We used aqueous cholesterol standards (Preciset Cholesterol, no. 125512; Bio-Dynamics/bmc) for three-point calibration of the aca HDLC and TC methods. One-point calibration of Dow HDLC and TC methods was done with the Dow cholesterol standard (included in Dow reagent kit no. 46550).

* * * *

Serum pools. Eighteen vials each of three serum pools were received frozen from the Centers for Disease Control (CDC), Atlanta, GA 30333. The HDLC reference values were 0.265, 0.351, and 0.501 g/L, respectively. Each day of analysis we allowed three vials of each serum pool to thaw

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1 Nonstandard abbreviations: HDLC, high-density lipoprotein cholesterol; TC, total cholesterol; CDC, Centers for Disease Control.

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at room temperature, mixed well, and analyzed the contents of each vial in duplicate.

Methods. For the acx method we determined HDLC and TC with Du Pont reagents, test packs, and procedures (1). For the Dow method, we obtained reagent kits nos. 45264 (HDLC) and 46550 (TC) from Dow Diagnostics, Dow Chemical Co., Indianapolis, IN 46268, and followed the manufacturer's instructions (2).

Procedure

Aliquots of fresh patients' serum that were received in the laboratory for routine analysis were used in this study. Icteric, hemolyzed, and lipemic sera were excluded. Paired aliquots of serum samples were analyzed simultaneously by both acx and Dow methods. All HDLC measurements were made in duplicate. The day-to-day precision for the acx and Dow HDLC methods was estimated by analyzing, in duplicate, three control sera during a period of three months (n = 22). The within-day precision for the acx method was estimated by 20 replicate determinations with the same three control sera.

Other Studies

Effect of hemoglobin. A blood sample from an apparently healthy ambulatory individual was collected into two tubes (10 mL each). We prepared hemolyzed serum from the blood in one tube, by a freeze–thaw cycle. The separated hemolyzed serum was assayed for hemoglobin; the nonhemolyzed serum separated from the second tube was assayed for HDLC.

 Serum samples with final hemoglobin concentrations ranging from 1 to 10 g/L were prepared by mixing the hemolyzed and nonhemolyzed sera. The samples were assayed for HDLC and the results expressed as the percentage recovered (nonhemolyzed serum HDLC = 100%). All measurements were made in duplicate.

Effect of bilirubin. A nonicteric serum pool (total bilirubin 5 mg/dL) was mixed with aqueous bilirubin to obtain samples with final bilirubin concentrations ranging from 10 to 250 mg/dL. A corresponding blank sample was prepared by mixing the serum pool with blank bilirubin. All samples were assayed for HDLC and total bilirubin. The percent recovery of HDLC from icteric samples was calculated by using the HDLC value of each corresponding blank as 100%. All measurements were made in duplicate.

Effect of lipemia. A lipemic serum pool (triglycerides 94.5 g/L, TC 11.3 g/L) was prepared by collecting the upper fatty concentrate obtained by centrifugation of lipemic specimens with an Airfuge® ultracentrifuge (Beckman Instruments, Inc., Spinco Division, Palo Alto, CA 94304). A nonlipemic serum pool (triglycerides 1.42 g/L, HDLC 0.454 g/L) was mixed with the lipemic serum pool to obtain lipemic samples with final triglyceride concentrations up to 11.8 g/L. The lipemic samples were assayed for HDLC and the results expressed as the percentage recovered (nonlipemic serum pool HDLC = 100%). All measurements were made in duplicate. The lipoprotein composition of the supernates was determined by lipoprotein electrophoresis (3).

Statistical analysis. We analyzed the data by de-biased linear regression according to Cornbleet and Gochman (4).

Results and Discussion

A comparison of the results between the acx (y) and Dow (x) methods for the determination of HDLC and TC in serum from 113 patients gave the following results: for HDLC, y = 1.17x − 0.007 g/L (Sx = 0.054, r = 0.944); for TC, y = 1.03x + 0.08 g/L (Sx = 0.13, r = 0.946). When the data were analyzed by a paired sample t-test, the acx results for HDLC and TC were significantly greater (p < 0.0001) than the Dow results. The proportional bias from the regression analyses between HDLC methods is greater than between the TC methods, 17% and 3%, respectively, which suggests that the observed difference between the two HDLC methods is due to a difference in the precipitation methods.

The specificity of lipoprotein precipitation by dextran sulfate/Mn2+ varies with the molecular size of dextran sulfate. Warnick et al. (5) found that separation with 50 000-Da dextran sulfate was similar to that obtained with heparin/Mn2+. A smaller Mr, dextran sulfate incompletely precipitated VLDL and LDL cholesterol, whereas a larger size precipitated some HDLC. Given these findings, the Dow HDLC method (dextran sulfate, Mr, 150 000) would be expected to underestimate HDLC.

To assess the correlation between the acx and the heparin/Mn2+ methods, we analyzed three CDC serum pools having HDLC reference values determined by heparin/Mn2+ precipitation method and by the CDC reference cholesterol method (modified Abell–Kendall procedure) (6). The differences between the reference and the mean measured HDLC concentrations in the three sera were 0.013, 0.035, and 0.034 g/L, respectively, with corresponding analytical recoveries of 104.9%, 110.0%, and 106.7%. Therefore, in comparison with the heparin/Mn2+ method, the acx method overestimates HDLC.

The day-to-day precision for the acx and Dow HDLC methods was essentially the same (Table 1). The within-day CV was less than 3% for three different control materials. The day-to-day precision for the acx method includes two calibrations of the instrument and therefore is substantially greater than the within-day precision.

In evaluating the interference by bilirubin, hemoglobin, and lipemia with the acx HDLC method, we found that bilirubin concentrations up to 170 mg/L and hemoglobin concentrations up to 4.3 g/L did not significantly alter the result for HDLC in serum. Lipemic specimens with triglyceride concentrations up to 5.43 g/L had clear supernates after treatment with the phosphotungstate precipitating reagent; the HDLC recovered from these supernates was between 98.5% and 106.9%. Lipemic specimens containing a triglyceride concentration greater than 5.43 g/L showed a broad β band on lipoprotein electrophoresis, indicating incomplete precipitation of the LDL and VLDL fractions. Thus, lipemic specimens with a triglyceride concentration greater than 5.4 g/L should not be assayed for HDLC with the acx method.

Table 1. Precision of the Du Pont acx and Dow HDLC Methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Precision</th>
<th>Control materials</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td>Within run (n = 22)</td>
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<tr>
<td>acx Mean, g/L</td>
<td>0.206</td>
<td>0.388</td>
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<tr>
<td>SD, g/L</td>
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<td>0.007</td>
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<tr>
<td>CV, %</td>
<td>2.9</td>
<td>1.8</td>
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<tr>
<td>Day to day (n = 20)</td>
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<tr>
<td>acx Mean, g/L</td>
<td>0.209</td>
<td>0.381</td>
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<tr>
<td>SD, g/L</td>
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<td>0.038</td>
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<td>CV, %</td>
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<td>10.0</td>
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<tr>
<td>Dow Mean, g/L</td>
<td>0.228</td>
<td>0.379</td>
</tr>
<tr>
<td>SD, g/L</td>
<td>0.021</td>
<td>0.037</td>
</tr>
<tr>
<td>CV, %</td>
<td>9.2</td>
<td>9.8</td>
</tr>
</tbody>
</table>

References

1. acx II/III test methodology: High density lipoprotein cholesterol (PN 704728C, 1/14/81) and cholesterol (PN 703705D, 2/19/81), Du
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Liquid and Lyophilized Quality-Control Materials Compared for Use in Continuous-Flow Analysis

Ronald J. Ellin and Betty A. Gray

Using a Technicon SMAC continuous-flow instrument, we compared a liquid quality-control material ("Decision level 3," Beckman) with two different lyophilized quality-control materials. The variances for 19 analytes in the three materials were significantly greater for Decision than for the Ortho Abnormal and the Omega II lyophilized control sera (p < 0.00001 for each comparison by the omnibus test). The precision for Decision was not due to incomplete mixing or carryover. The viscosity of Decision, however, was more than twice that of the two lyophilized control sera or of pooled human serum.

Frozen pooled serum, lyophilized serum, and liquid serum with an additive are the three major products used as quality-control sera. The most recently developed quality-control material is "Decision" (Beckman Instruments, Inc., Fullerton, CA 92634), a clear, homogeneous pooled serum stabilized with 330 mL of ethylene glycol per liter (1). We evaluated the precision of analyses when this material was used in a SMAC continuous-flow analyzer (Technicon Instruments, Tarrytown, NY 10591), and compared these results with those obtained with two lyophilized control sera.

Materials and Methods

Apparatus

We used Technicon-recommended methodologies and reagents. For one part of the study, we modified one of our two SMACs by adding a five-turn mixing coil (part no. 178-G196-01) before and after the pre-dilution cartridge, to assess the effect of additional mixing on precision.

To measure viscosity (five determinations with each specimen) we used a size-75 routine viscometer (no. CFRC; Cannon-Fenske, State College, PA 16801), kept in a constant-temperature water bath at 37 °C.

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Control Sera

Decision level 3 (lot no. C012234, compared with Ortho abnormal in Table 1) was shipped by the manufacturer on solid CO2—which had sublimed by the time the shipment was received, although the product was still cold to the touch. The product was immediately stored at -20 °C. The manufacturer, queried about the warming of the product, indicated that no damage had occurred and that the product was suitable for use. A second lot of Decision level 3 (lot no. C104033, compared with Omega II in Table 1) was obtained with solid CO2 still present in the shipping container and immediately stored at -20 °C. The Decision level 3 was packaged in 20-mL plastic squeeze bottles, which were stored at -20 °C until used in this study, at which point it was kept in a refrigerator at 2 to 8 °C, for no longer than six days.

The two lyophilized control sera studied were Ortho abnormal (lot no. 107564; Ortho Diagnostic Systems, Inc., Raritan, NJ 08869) and Omega II (lot no. 4822F001B; Hyland Laboratories, Malvern, PA 19355), reconstituted with the diluent supplied by the manufacturers, and according to their instructions.

Procedures

Each control sera was analyzed five times per day for 20 (Decision vs Ortho) or 16 (Decision vs Omega) working days. The control sera were placed in consecutive cups in alternating order for analysis by SMAC.

The effect of carryover and additional mixing on the precision of Decision level 3 was also evaluated. For carryover, Decision level 3 was placed in two consecutive cups and analyzed five times per day for 15 working days. To assess the effect of additional mixing, one of our two SMAC instruments was modified by adding two five-turn mixing coils and Decision level 3 was analyzed five times per day for 15 working days on both SMAC instruments.

Total imprecision was assessed by calculating the variance and coefficient of variation (CV) for each analyte among the three control materials. The p value for the F ratio (variance for Decision divided by the variance for the lyophilized control material) was obtained by use of pro-