Overestimation of HDL-Cholesterol Using a Homogeneous “Direct” Assay

Wei Zhao,¹ Carolyn Chaffin,¹ Renee A. Desmond,² Bruce Hodges,¹ Thomas M. Daly,¹ C. Andrew Robinson,¹ and Robert W. Hardy^{1*}

¹Department of Pathology, Division of Laboratory Medicine, University of Alabama at Birmingham, Birmingham, Alabama

²Department of Medicine, Medical Statistics Division, University of Alabama at Birmingham, Birmingham, Alabama

Direct HDL-cholesterol (HDLc) assays have several advantages over other assays that are more laborious and time-consuming. A recent College of American Pathologists (CAP) report indicates that at least 385 Beckman LX-20 analyzers use the selective detergent (homogeneous or direct) method for analyzing HDLc. There is no published evaluation of direct HDLc assays on the Beckman platform. Here we report our evaluation of a direct HDLc assay marketed by Beckman for the Beckman LX-20 analyzers. In general, the assay

performed well; however, the total error was not within National Cholesterol Education Program (NCEP) guidelines. This was largely because of a significant positive bias that appeared to be due, at least in part, to triglycerides. This bias was worse at HDLc concentrations <40 mg/dL, which may make it difficult to identify a low HDLc concentration and consequently an increased risk for cardiovascular disease (CVD). *J. Clin. Lab. Anal.* 18:42–44, 2004. © 2004 Wiley-Liss, Inc.

Key words: triglycerides; correlation; total error; Beckman LX20; ultracentrifugation

INTRODUCTION

HDL-cholesterol (HDLc) is an established lipoprotein risk factor for cardiovascular disease (CVD), and can be measured by direct methods. These methods offer several potential advantages over ultracentrifugation, including speed (they are easily automated) and reduced cost. Warnick et al. (1) recently reviewed HDLc measurement methods. In general, direct assays have received favorable reviews; however, the direct HDLc adaptation of the N-geneousTM assay to the Beckman LX20 (Beckman-Coulter, Brea, CA) has not yet been evaluated. A recent CAP report indicates that at least 385 Beckman LX-20 analyzers use the selective detergent (direct) method to analyze HDLc.

Patient Samples

For this study, we collected 77 fasting-patient samples (EDTA plasma) that had triglyceride (TG) concentrations of 200–1,200 mg/dL. Fourteen of these samples had a TG concentration greater than 400 mg/dL. The plasma was separated and measured the next day (within 48 hr) by direct assay at the University of

Alabama at Birmingham Hospital chemistry laboratory, and by reference assay at Atherotech (Birmingham, AL).

HDLc Analysis

The University of Alabama at Birmingham Hospital chemistry laboratory measured HDLc using the Beckman Synchron LX (Beckman-Coulter) N-geneousTM direct method. HDLc was measured with N-geneousTM reagents adapted for the Beckman Synchron LX, which uses a detergent that solubilizes only HDL particles, thereby allowing HDLc to be measured by a color-producing enzymatic reaction.

Atherotech measures HDLc by a vertical auto profile (VAP) technique that uses density gradient ultracentrifugation to separate lipoproteins, and subsequently

*Correspondence to: Robert W. Hardy, Department of Pathology, Division of Laboratory Medicine, University of Alabama at Birmingham, 701 South 19th St., LHRB 531, Birmingham, AL 35294-0007. E-mail: hardy@path.uab.edu

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Fig. 1. Comparison of the direct assay with the VAP reference method in 77 patients.

measures cholesterol in each of the resulting density bands (2). The results from this assay are comparable to those from a CDC-standardized laboratory (3).

Statistical Analysis

Descriptive statistics were calculated for HDLc and stratified by laboratory. A Shapiro-Wilk statistic was used in combination with normal probability plots to assess the distribution of HDLc. The mean percentage bias (mean difference from values obtained via direct methods and referent values expressed as a percentage of the referent value), standard error of the percentage differences, and limits of agreement (mean percentage difference ± 2 SD) were calculated using the VAP HDLc value as the reference value. To determine the correlation between the two methods, a plot of the direct vs. reference method was made, and the relationship was evaluated by linear regression. To evaluate the source of the bias, the HDLc percentage bias measurements were used as the responses. Significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

The HDLc assay demonstrated good correlation with the VAP HDLc results (Fig. 1, $y = 0.9081x + 6.8376$, $R^2 = 0.7702$, $SEE = 5.2$, $n = 77$). In addition, our be-

tween-day precision (30 days using serum-based lipid controls (Bio-Rad, Richmond, CA)) demonstrated a 3.7% and 3.5% CV at 24 mg/dL and 77 mg/dL, respectively. Linearity was confirmed using seven Casco linearity standards ranging from 0 to 149 mg/dL, each run in quadruplicate. A plot of the resulting means yielded a line with a slope of 1.07, intercept of -1.03 , $R^2 = 0.998$, and $SEE = 2.4$. Interference studies indicated a 3% and 12% negative interference at 300 and 500 mg/dL hemoglobin (4 points run in total with 500 mg/dL maximum), respectively; 0% and 6% negative interference at 7.5 and 12.4 mg/dL bilirubin (6 points run in total with 12.4 mg/dl maximum), respectively; and 5% and 13% negative bias at 560 and 2240 mg/dL triglyceride (from intralipid, 6 points run with 2,240 mg/dL maximum), respectively.

The patient-sample correlation results indicated that there was an overall significant positive bias (9.4%, $P = 0.0001$, 95% confidence interval, 6.02–12.87%) compared to the VAP. Since many of the samples showed elevated triglyceride concentrations, we plotted the percent difference between the two methods against triglyceride concentration and found that there was a significant positive relationship ($P = 0.013$). However, the slope is small and the R^2 value is low ($y = 0.0192x + 3.811$, $R^2 = 0.0841$). Nevertheless, when

the percent difference between the two methods was plotted against the VAP-LDLc concentrations, there was no significant relationship ($P=0.24$). Thus it is likely that triglyceride concentrations account, at least in part, for the positive bias observed with the direct HDLc method. Using the NCEP guidelines for calculating total error (total error = absolute value [mean bias%] + 1.96 [mean imprecision%]), we found a 16.6% total error for the direct HDLc assay. This is higher than the NCEP recommendation of $\leq 13\%$. However, patient samples with HDLc >39 mg/dL had a total error of 11% ($n=40$), which is within the recommended guidelines, compared to 22% for those with HDLc <40 mg/dL ($n=38$). These data are somewhat surprising given the overall favorable reviews of the direct method (1), and an evaluation that found the direct method well within NCEP guidelines when performed on another platform (4). It is possible that the different platform and reagent/calibration formulations used account for this difference.

These data indicate that bias is greater at low concentrations of HDLc; thus, the main danger with

the direct HDLc assay is that potentially low HDLc values may be overestimated. This could have important clinical implications, given the significance of a low HDLc and the association of high triglycerides with low HDLc.

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