Accuracy and Precision of HDL Cholesterol Measurements Using an Office Chemistry Analyzer

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Background. A physician can obtain a patient's complete lipoprotein profile at the time of the office visit including assays of the total serum cholesterol, high-density lipoprotein cholesterol (HDL-C), and fasting triglyceride concentrations, and then calculate the low-density lipoprotein cholesterol (LDL-C). Until recently, this was not possible. Instruments are currently available that provide reliable rapid total serum cholesterol and fasting triglyceride measurements.

Methods. This study evaluated the accuracy and precision of a recently developed analytical method for the rapid measurement of HDL-C (Seralyzer Cholesterol System) as compared with a reference clinical laboratory method (Kodak Ektachem 700 XR). Blood specimens were taken from 90 participants and were analyzed in duplicate for HDL-C concentrations and total cholesterol using the Seralyzer and a standard Ektachem 700 XR.

Results. Nearly all (98.9%) of the initial Seralyzer HDL-C measures were within ±0.08 mmol/L (±3 mg/dL) of the duplicate Seralyzer values. Most (98.3%) of the Seralyzer HDL-C results were within ±0.16 mmol/L (±6 mg/dL) of the Kodak HDL-C values.

Conclusions. The Seralyzer HDL-C test provides a reliable and accurate measure of the HDL-C concentration.

Key words. Lipoproteins, HDL cholesterol; diagnosis; laboratory. *J Fam Pract 1991; 32:382-386.*

The National Cholesterol Education Program (NCEP) was inaugurated in 1985 by the National Heart, Lung, and Blood Institute of the National Institutes of Health in response, at least in part, to the findings of several major epidemiological and clinical studies that presented strong evidence of a causal link between high serum cholesterol concentrations and coronary artery disease.[1–5] Three major classes of lipoprotein-bound cholesterol play a part in the development of coronary artery disease. There is a direct relationship between low-density lipoprotein cholesterol (LDL-C) and the risk of developing coronary artery disease, and an inverse relationship between high-density lipoprotein cholesterol (HDL-C) and the risk of developing coronary artery disease.[1,4–6] In addition to these causal relationships, the Lipid Research Clinics Coronary Primary Prevention Trial[7,8] recently provided evidence that lowering the LDL-C also lowers the incidence of coronary artery disease. The drug cholestyramine and a moderate cholesterol-lowering diet reduced both the level of serum LDL-C concentration and the incidence of coronary artery disease.

Since the inception of the NCEP, great progress has been made in the detection, evaluation, and treatment of hypercholesterolemia. The 1988 NCEP report suggested two approaches to lower blood-cholesterol concentrations.[9] The patient-based approach seeks to identify high-risk persons in need of intensive intervention efforts. NCEP recommended that all adults over 20 years of age have their total serum cholesterol measured at least every 5 years and that those with borderline-high (5.17 to 6.18 mmol/L, or 200 to 239 mg/dL) or high (≥6.20 mmol/L, or ≥240 mg/dL) serum cholesterol levels have their cholesterol level measured more often. Persons with a high serum cholesterol level or individuals with borderline-high serum cholesterol levels and two other risk factors for developing coronary artery disease, such as hypertension and smoking, are advised to have a lipoprotein analysis done to provide a more precise estimate of their degree of risk. Such an analysis provides the fasting concentrations of total cholesterol, high-density lipoprotein cholesterol, and total triglycerides. Using this

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information, the LDL-C can be calculated in mmol/L (ie, SI units):[9]

LDL-C = total cholesterol − HDL-C

− fasting triglyceride/2.18

This can also be calculated in mg/dL:

LDL-C = total cholesterol − HDL-C

− fasting triglyceride/5

The above equation is valid for triglycerides up to 4.52 mmol/L (400 mg/dL). The NCEP considers a low level of HDL-C (<0.91 mmol/L, or <35 mg/dL) to be a risk factor for coronary artery disease.

The total serum cholesterol concentration is a good initial measure for identifying high-risk individuals. Currently there are fast, accurate in-office tests available that can determine the total serum cholesterol and allow the physician to make some decisions about further testing and treatment while the patient is still in the office. Until recently, fast, accurate, in-office tests for LDL-C or HDL-C concentrations have not been available. If the total serum cholesterol, HDL-C, and fasting triglyceride concentrations are all determined at the time of the office visit, the LDL-C concentration can also be calculated at that time using the above equation. With this information in hand, the physician can counsel the patient concerning his or her cholesterol profile at the time of the office visit and make arrangements for follow-up confirming tests if they are warranted. This rapid feedback avoids the problems physicians encounter when they attempt to contact patients with their final recommendations at a later date. A rapid in-office test for determining total cholesterol and HDL-C concentrations provides this immediate feedback.

The purpose of this study was to determine the accuracy and reliability of the Seralyzer instrument (Seralyzer Cholesterol System, Diagnostics Division of Miles, Elkhart, Ind). In this report, accuracy refers to how close the measure is to the true value. Reliability or precision refers to how repeatable results are on the same instrument for a given specimen.

Methods

Ninety patients and employees from The Ohio State University Family Practice Center were recruited in the spring of 1989 for this study. The participants were between the ages of 20 and 65 years, and an equal number of men and women were in the group.

A nonfasting blood specimen was taken from each participant. The total cholesterol and HDL-C concentration were determined in duplicate, in accordance with the manufacturer's instructions. For most (74) of the participants, these procedures were performed by an ASCP-certified medical technologist. In addition, the serum specimens were assayed in the University Hospital's clinical chemistry laboratory for HDL-C concentrations in duplicate using an Ektachem 700 XR instrument and following Kodak's recommended precipitation procedure (Eastman Kodak Co, Rochester, NY).

The Seralyzer HDL-C results were analyzed to examine their reproducibility and accuracy compared with that of the standard Kodak Ektachem 700 XR HDL-C test results.

Results

The Seralyzer HDL-C test results were highly correlated (r = .97) with the Kodak Ektachem 700 XR test results (Figure 1). There were no substantial differences between the HDL-C results of the two instruments. The Kodak HDL-C values ranged from 0.54 to 1.85 mmol/L (21.0 to 71.5 mg/dL), while the Seralyzer HDL-C values ranged from 0.41 to 1.80 mmol/L (15.8 to 69.1 mg/dL).

Table 1 shows the frequency distribution of the differences between the Seralyzer initial values and the duplicate values for HDL-C. Most of the results of the duplicate tests (98.9%) were within ±0.08 mmol/L (±3 mg/dL) of their corresponding initial results. All duplicate test results were within ±0.10 mmol/L (±4 mg/dL). Most (88.6%) of the HDL-C results had a coefficient of variation of the replicates of 3% or less, and 98.7% had 5% or less.

Table 2 is the frequency distribution of the differences between the Seralyzer and the reference HDL-C results. Over half of the Seralyzer test results (54.2%) were within ±0.05 mmol/L (±2 mg/dL) of the reference values, nearly all (98.3%) were within ±0.16 mmol/L (±6 mg/dL), and all were within ±0.21 mmol/L (±8 mg/dL). Additionally, 91.6% of all of the Seralyzer HDL-C values were within ±0.13 mmol/L (±5 mg/dL) of the reference results. For Seralyzer HDL-L values of 1.29 mmol/L (50 mg/dL) or lower, 91.3% were within ±0.13 mmol/L (±5 mg/dL) of the reference values.

Discussion

The second NCEP expert panel, the Laboratory Standardization Panel (LSP) for cholesterol measurement,[10] recommended that the overall precision or reproducibility of an instrument for measuring total cholesterol

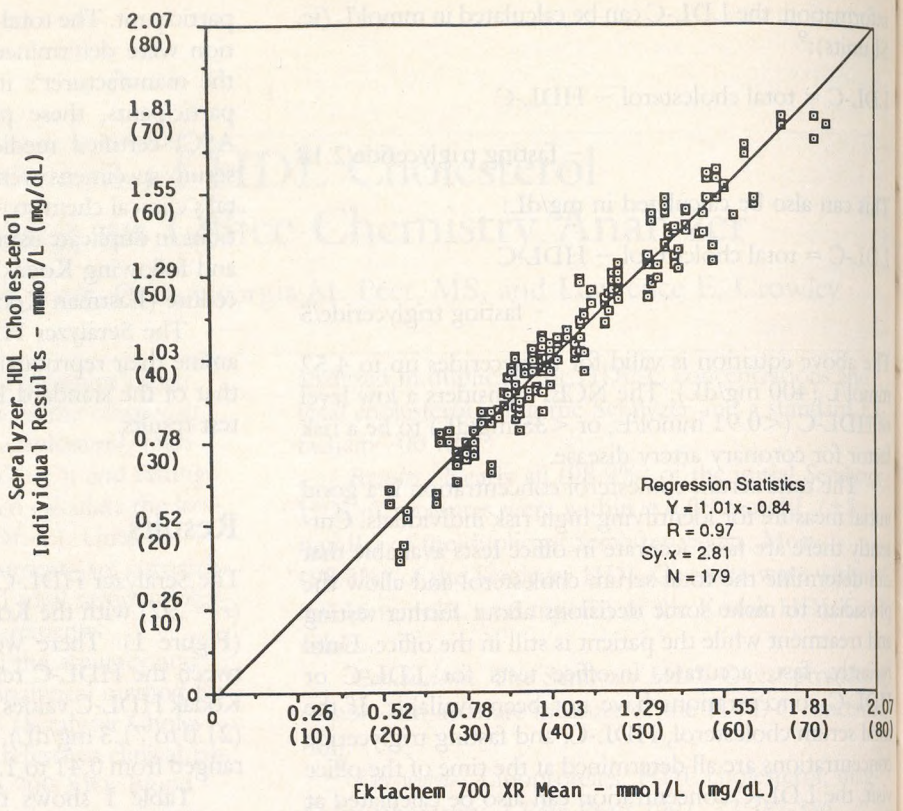

Figure 1. Regression plot of duplicate values for high-density lipoprotein cholesterol (HDL-C) concentrations by the Seralyzer Cholesterol System and Ektachem 700 XR Methods.

should be within a 5% coefficient of variation with an ultimate goal of 3% or less; similar recommendations were made for the measurement of HDL-C. The Seralyzer was reliable in its assay of HDL-C as evidenced by the fact that 88.6% of the values had a 3% coefficient of variation or less, and 98.7% had a 5% coefficient of variation or less. Naito[11] and Cooper et al[12] report that precision is not the major problem in measuring HDL-C concentrations; rather, the problem is one of accuracy or bias from the true value.

The LSP also recommends that deviations from the true value should not exceed 5%; the ultimate goal in the measurement of total cholesterol and HDL-C is for deviation not to exceed 3%.[10] Cooper et al question the soundness of such standards for the measurement of HDL-C in light of their study, which found that HDL-C has a mean within-person total coefficient of variation of 4.5% daily, 7.7% monthly, and 8.4% yearly.[12] It should be noted here that a 5% bias for a total cholesterol concentration of 5.17 mmol/L (200 mg/dL) allows a range of 4.91 to 5.43 mmol/L (190 to 210 mg/dL). A 5% bias for an HDL-C concentration of 0.90 mmol/L (35 mg/dL) allows a range of 0.86 to 0.95 mmol/L (33.3 to 36.8 mg/dL). Levy[13] emphasized the need for accuracy when HDL-C results are at 1.29 mmol/L (50 mg/dL) and below. He suggested a maximal bias of ±0.13 mmol/L (±5 mg/dL) from which to determine risk. Naito indicated that many clinical laboratories (36.5% to 52%) are not measuring HDL-C accurately enough (a conclusion based on previous and current laboratory surveys); their test results often exceed the true value or

Table 1. Distribution of Differences Between Initial and Duplicate Measures of High-Density Lipoprotein Cholesterol Concentrations by the Seralyzer

Magnitude of Difference mmol/L (mg/dL)	No.	Percent
±0.03 (±1.0)	47	52.8
±0.05 (±2)	76	85.4
±0.08 (±3)	88	98.9

Table 2. Distribution of Differences Between Duplicate Measures of High-Density Lipoprotein Cholesterol Concentrations by Seralyzer and Reference Method*

Magnitude of Difference mmol/L (mg/dL)	No.	Percent
±0.05 (±2)	97	54.2
±0.10 (±4)	149	83.2
±0.16 (±6)	176	98.3

**Kodak Ektachem 700 XR.*

overall mean by 10% or more.[11] He believes the problem is the variability in procedures for HDL-C separation and cholesterol analysis. Most (91.3%) of the Seralyzer HDL-C measures met Levy's standards. It is important to remember that the biases reported here are not based on known values, but rather on the results provided by a single hospital laboratory method. The interlaboratory precision (coefficient of variation) of the standard Kodak method for the measurement of total cholesterol is 3.3% at 5.17 mmol/L (200 mg/dL) and 3.1% at 6.20 mmol/L (240 mg/dL).[14]

The NCEP recently clarified their recommendations concerning HDL-C screening.[15] Individuals with borderline-high (200 to 239 mg/dL) serum cholesterol and one coronary artery disease risk factor should have their HDL-C concentration determined, since an HDL-C concentration of <0.91 mmol/L (<35 mg/dL) constitutes an additional risk factor for coronary artery disease. Persons with high (≥6.20 mmol/L or ≥240 mg/dL) serum cholesterol should undergo further lipoprotein analysis regardless of whether they have other coronary artery disease risk factors. Repeat determinations of borderline high cholesterol and HDL-C values are appropriate owing to within-person and within-laboratory variability.

Sempos et al[16] estimated that 41% of adults should have further lipoprotein analysis following the initial measurement of their total serum cholesterol, and that 36% of adults are candidates for medical advice and intervention. The Seralyzer HDL-C test kit would allow the physician to make a decision concerning a patient's need for advice and intervention or for further testing at the time of the office visit. If a fasting triglyceride concentration is also determined at the time of the visit and it is below 4.52 mmol/L (400 mg/dL), the LDL-C can be calculated using the formula previously discussed. The physician would then have a complete lipoprotein profile from which to make a decision. This is important because definitive therapy is based on the LDL-C level, not just on the total cholesterol level.

The NCEP did not recommend that all adults be screened for their HDL-C concentrations.[15] One reason for this was the added time, cost, and inconvenience of additional testing. These factors, however, can be minimized by in-office tests, since the HDL-C test can be performed simultaneously with the other cholesterol tests. In long-term monitoring of cholesterol for high-risk individuals, in-office tests for HDL-C would save time and money for both the patient and physician. If physicians do not choose to screen patients' HDL-C levels, they can still use this test in follow-up evaluations of patients.

It is critical at this point to stress the importance of having trained laboratory personnel performing these tests. The Laboratory Standardization Panel states that technical personnel must be properly trained in the use and maintenance of portable chemistry analyzers and in the proper use of quality-assurance procedures.[10] There are at least two procedural steps in using the Seralyzer for HDL-C testing that require careful attention. Testing conducted by certified laboratory technologists will probably provide the most reliable and accurate results. It is also important to reinforce the NCEP recommendation that borderline and high-risk individuals have repeated cholesterol measurements over time; single values can be misleading.[9] Any measurement of total cholesterol or HDL-C that is at or near the defined borderline-high or high-risk values should be repeated as part of the evaluation and monitoring process.

The Seralyzer HDL-C test provides a reliable and accurate measure of the HDL-C concentration. When the test is performed by a certified laboratory technologist, it is as accurate as many of the clinical laboratory instruments currently in operation.

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