

Standardization of Lipid Tests

Background

The National Cholesterol Education Program (NCEP) of the National Institutes of Health (NIH) has made a concerted effort to identify and treat every American adult at increased risk for coronary heart disease because of high blood cholesterol.

As part of this effort, the Adult Treatment Panel (ATP) of the NCEP has established clinical decision points and treatment guidelines that require reliable measurements of lipids that include total cholesterol (TC), triglycerides, low-density lipoprotein cholesterol (LDL) and high-density lipoprotein cholesterol (HDL).

Because of the importance of lipid test results in the assessment and management of coronary heart disease risk, the NCEP established the Laboratory Standardization Panel (LSP) on Blood Cholesterol Measurement. This panel is tasked with assessing the reliability of cholesterol measurement in clinical laboratories, and recommending means to improve the precision and accuracy of cholesterol testing. In 1988, the LSP recommended that total cholesterol measurements be standardized so that values would be traceable to the Centers for Disease Control and Prevention (CDC) reference method (modified Abell-Kendall) or the National Institute of Standards and Technology (NIST) definitive method (isotope dilution-mass spectrometry).

Standardization

Since there are many valid methods and instruments for performing lipid analyses, it is not practical to require that all laboratories use the same method, calibrators, and controls. Instead the LSP has established the

basis of standardization to a defined common accuracy base (traceability). The major components of this accuracy base comprise a hierarchy of methods and materials: a definitive method and primary reference materials; a reference method and secondary reference materials.

For each lipid (TC, HDL, TRG), an analytical method must be adopted and widely accepted as the basis for universal reference. Though the selected definitive method establishes the true value for an analyte, it can be expensive, cumbersome and not readily available. Because of such limitations, a definitive method is primarily used to validate a more widely available reference method developed for broader use and application. This reference method can then be used in turn by the testing community; transferring the accuracy base yet again to a broader base. This is done through the use of reference materials.

Through the LSP, reference methods and materials have been developed for TC, and an effort has been made to standardize TC measurement throughout the US. The CDC has recently instituted a standardization program for HDL, and one is being developed for triglycerides.

Cholesterol

The standard for accuracy in cholesterol measurement is the National Reference System for Cholesterol established by the National Committee for Clinical Laboratory Standards (NCCLS). It is made up of the National Institute of Standards and Technology definitive method, the CDC reference method, a certified pure cholesterol standard (NIST SRM 911b), and certified serum-based

secondary reference materials from CDC and College of American Pathologists (CAP).

Both the definitive and reference methods are calibrated using the NIST SRM 911b pure cholesterol material. The two methods are monitored on an ongoing basis. A recent comparison showed correlation of the reference method within 1.6% of the definitive method.

The total cholesterol method on the Cholestech LDX test cassettes is calibrated to reference methods traceable to the CDC reference method.

HDL-Cholesterol

The second report of the NCEP's Adult Treatment panel brought attention to HDL cholesterol as a coronary heart disease (CHD) risk factor. In addition it recommended the measurement of HDL, along with total cholesterol, for use in the detection, evaluation and treatment of individuals who may be at increased risk for CHD. Lipoproteins such as HDL are a heterogeneous mixture of lipids and proteins and are not easily defined. As a result, significant overlap can exist in the properties of lipoproteins.

The measurement of HDL is complicated by the fact that there are biases between the various precipitating reagents used to separate low density lipoprotein cholesterol (LDLC) and very low density lipoprotein cholesterol (VLDL) from the HDLC. In addition the matrix characteristics of calibrators cause significant biases when analyzing fresh patient specimens.

The reference method for HDL used at CDC is a complicated three-step

procedure that combines removal of VLDL by ultracentrifugation, precipitation of LDL by heparin-manganese, and cholesterol analysis of the HDL-containing liquid by the CDC Abell-Kendall reference method.

The major problems with the CDC HDL reference method are technical difficulty, large sample volume requirements, and prolonged test completion time. It also requires the use of an ultracentrifuge, which is very expensive and difficult to operate. This makes widespread use of this method for routine standardization of HDL methods impractical. However, since this HDL method has served as the reference method for the CDC-NHLBI investigations from which coronary heart disease risk estimations and population distributions have been derived, there is justification for continuing to use this procedure as a national reference method. For this reason the Cholesterol Reference Method Laboratory Network (CRMLN) has developed and evaluated a designated comparison method (DCM) which is now available to help manufacturers in the standardization of their HDL cholesterol methods.

Several field methods for HDL testing are in use at the present time. Most of these methods depend on chemical precipitation of non-HDL cholesterol (LDLC and VLDL). The precipitate is removed by low speed centrifugation and the HDL is measured in the remaining liquid. Though these methods are similar in technique, they differ somewhat in their ability to precipitate lipoproteins, and results on different methods may not be equivalent. In addition a number of direct HDL cholesterol methods have been developed recently which eliminate the precipitation step and allow complete automation of the HDL assay. As a result of these different methodologies, there can be significant variability between different methods in the field.

The HDL method incorporated on the Cholestech LDX test cassettes is calibrated to reference methods that

are traceable to the CDC reference method.

LDL-Cholesterol

The determination of LDL is important as LDL continues to be the primary target of cholesterol-lowering therapy. The process of establishing an accuracy target for LDL is complicated for the same reasons as HDL. The CDC has adopted a variation of a multi-step beta quantification procedure used by the Lipid Research Clinics, which combines separation by ultracentrifugation and chemical precipitation, as a reference procedure for LDL. The CDC reference value for LDL is calculated as the difference in cholesterol between the measured HDL and the cholesterol recovered in a certain fraction obtained by ultracentrifugation. Several methods have recently been developed for the direct measurement of LDL and are available for clinical use. The most frequently used method for determining LDL is to calculate an estimate of it using the Friedewald equation ($LDL = TC - HDL - Triglycerides/5$). The Cholestech LDX determines LDL levels using this formula.

Triglycerides

Triglycerides are not a distinct molecule, but a heterogeneous group of similar compounds. A definitive method for measuring triglycerides is being developed. A reference method for triglycerides was established at CDC in 1963 and has been used since as the CDC's accuracy base for standardization programs. The method is very exacting and complicated. The CDC presently uses a triolein/tripalmitin (two different triglycerides in combination) standard to reflect the average unsaturated/saturated triglyceride composition in human serum. A tripalmitin standard is available from NIST, but is insoluble in water and is therefore unsuitable for enzymatic methods. The CDC is developing a secondary reference method that will be used to standardize field methods. In the mean time, considerable variability in methods will continue to be seen in the field. The Cholestech

LDX triglycerides method is calibrated against a CDC standardized enzymatic method.

Performance Criteria

The role of cholesterol and other lipids in the assessment of CHD has accentuated the need for more precise and accurate laboratory measurements. To assess the quality of results in individual laboratories, meaningful criteria to evaluate their precision and accuracy are needed. In 1988, realizing this need, the LSP of the NCEP recommended interim and ideal laboratory goals for both bias and precision for total cholesterol measurement. The panel recommended that, as a US goal, clinical laboratories should by 1992 achieve a bias of <3% of the CDC reference method and an overall precision consistent with a CV of 3% or less. For a single analysis, the allowable total error (imprecision plus bias) would be $\pm 8.9\%$. This was one of the most important recommendations from the LSP, because it established, for the first time, specific performance criteria by which clinical laboratories could judge the reliability of cholesterol assays.

Recently, the CDC has recommended the following performance criteria for lipid tests based on total error.

| Analyte | Total Error |
|------------------------|--------------------|
| Total Cholesterol | $\leq 8.9\%$ |
| HDL Cholesterol (1998) | $\leq 13\%$ |
| Triglycerides | $\leq 15\%$ |

Matrix Effects of Reference Materials

The traditional approach to standardization has been to use reference materials and calibrators that have values assigned by the reference or definitive methods. This approach is based on the assumption that reference materials mimic fresh patient specimens when assayed on a variety of methods. However, several recent studies have indicated that significant differences in lipid test results are seen when some analytical

systems are compared to the reference processed serum materials. As a result of their preparation, the processed materials may undergo changes that affect their assay characteristics. Using the reference target values of these commercially prepared materials to calibrate a matrix-sensitive assay system has been shown to result in significant biases when analyzing fresh patient specimens. The use of frozen, fresh serum minimizes the problems associated with matrix interactions, but even the process of freezing appears to affect some analytical methods. Until reference materials free of matrix effects are available, the LSP recommends comparing the analytical system with the cholesterol reference method by split "fresh" patient specimens. All tests on the Cholestech LDX test cassettes are standardized with fresh patient specimens.

Resources Available for Standardization

To facilitate access to the NRS/CHOL by manufacturers and clinical laboratories, the CDC has established a national network of laboratories that use the cholesterol reference method. The Network Laboratories perform the Abell-Kendall procedure exactly as it is performed at CDC and are standardized to CDC through participation in the CDC-NHLBI Lipid Standardization Program.

Cholestech participates in this program, and our total cholesterol assay has been certified as traceable to the National Cholesterol Reference Method Laboratory Network. We plan to participate in standardization programs for HDL and triglycerides, when available.

A list of participating Network Laboratories with whom we have collaborated include:

- University of Washington
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Northwest Lipid Research Laboratories
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