

Improving Assessment of Cardiovascular Disease Risk Factors by Standardizing Manufacturers of Diagnostic Assays

M. Dasti^{1,2}

M.M. Kimberly²

G.L. Myers²

¹Battelle, Atlanta, GA

²Centers for Disease Control and Prevention, Atlanta, GA.

Abstract

Context

To correctly diagnosis and manage coronary artery disease, physicians need accurate, reliable lipid and lipoprotein measurements. The National Cholesterol Education Program (NCEP) recommended that these measurements be traceable to the Centers for Disease Control and Prevention (CDC) reference methods. More than 100,000 clinical laboratories are in the United States, which makes direct standardization by CDC impractical.

Objective

CDC established the Cholesterol Reference Method Laboratory Network (CRMLN) to provide traceability to reference methods. The CRMLN improves laboratory performance by working with manufacturers to evaluate the performance of clinical diagnostic products. This poster reports the certification activity of manufacturers.

Methods

To ensure accurate calibration, manufacturers must demonstrate acceptable performance of their assays on the measurement of human serum samples. To accomplish this, manufacturers perform a sample comparison with a CRMLN laboratory. Those that meet NCEP performance criteria for imprecision and inaccuracy receive a Certificate of Traceability. The data reported for manufacturers were gathered from November 2002 to November 2004.

Results

Manufacturers evaluated 72 methods for total cholesterol (TC); 83% of these were certified. Seventy-four high-density lipoprotein cholesterol (HDL-C) methods were evaluated; 68% were certified. Thirty-four low-density lipoprotein cholesterol (LDL-C) methods were evaluated; 82% were certified. Manufacturers that were certified had an average percent bias ranging from -2.9 to 2.1% for TC, from -4.4 to 5.4% for HDL-C, and from -3.5 to 2.8% for LDL-C. Manufacturers that were certified had an average percent coefficient of variation (CV) ranging from 0.3 to 2.5% for TC, from 0.4 to 4.0% for HDL-C, and from 0.7 to 3.3% for LDL-C.

Conclusions

By providing traceability for manufacturers, the CRMLN improves the reliability of cholesterol testing in clinical laboratories. This leads to improved patient care because the methods used by clinical laboratories are properly calibrated.

Introduction

The diagnosis and management of coronary artery disease require accurate lipid measurements. In 1988, the National Cholesterol Education Program (NCEP) Laboratory Standardization Panel recommended values traceable to the CDC's reference method (modified Abell-Kendall (AK) method) be used to classify patients according to medical decision points. The various methods developed by manufacturers and those used by clinical laboratories need to be traceable to this reference method. In 1995, the NCEP Working Group on Lipoprotein Measurement recommended that the CDC's reference methods serve as the accuracy base for HDL-C and LDL-C.

The Cholesterol Reference Method Laboratory Network (CRMLN) was established in 1989 to provide traceability of lipid and lipoprotein measurements to the CDC's reference methods. True and precision of CRMLN laboratories are evaluated in bimonthly performance surveys. CRMLN laboratories meet very strict performance criteria. CRMLN laboratories in turn certify manufacturers of clinical diagnostic products and clinical laboratories. This program results in reliable lipid and lipoprotein measurements to aid in the management of heart and vascular disease.

Health Risks of Hypercholesterolemia

Heart disease, the number one cause of death in America today, results in approximately 500,000 deaths per year. Over 6 million Americans suffer from atherosclerotic coronary artery disease. High cholesterol levels are associated with heart and vascular disease. In particular, high levels of LDL-C and low levels of HDL-C are associated with an increased risk for coronary artery disease as well as cerebrovascular disease. Studies have shown a reduction of TC and LDL-C as well as an increase in HDL-C reduces the risk of coronary artery disease. The NCEP developed medical decision points to determine which patients require further therapy for hypercholesterolemia.

NCEP ATP III Guidelines for Lipoproteins

ATP III Classification of Total, HDL and LDL Cholesterol (mg/dL)	
Total Cholesterol	
< 200	Desirable
200-239	Borderline High
≥ 240	High
HDL Cholesterol	
< 40	Low
≥ 60	Desirable
LDL Cholesterol - Primary Target of Therapy	
< 100	Optimal
100-129	Near optimal/above optimal
130-159	Borderline high
160-199	High
≥ 200	Very high

CRMLN Participants and the Reference Methods Performed

International

- Erasmus Medical Center, Rotterdam, The Netherlands: AK, HDL-C, HDL-C-DCM, LDL-C
- Osaka Medical Center for Health Science and Promotion, Osaka, Japan: AK, HDL-C, HDL-C-DCM, LDL-C
- Institute of Biochemistry, Glasgow, Great Britain: AK, HDL-C-DCM
- Canadian External Quality Assessment Laboratory, Vancouver, British Columbia, Canada: AK, HDL-C, HDL-C-DCM
- H.S. Raffaele, Milano, Italy: AK, HDL-C-DCM
- Fundacion Bioquimica Argentina, La Plata, Argentina: AK, HDL-C-DCM
- Beijing Institute of Geriatrics, Beijing, People's Republic of China: AK, HDL-C-DCM

Domestic

- Northwest Lipid Research Laboratories, Seattle, Washington: AK, HDL-C, HDL-C-DCM, LDL-C
- Wadsworth Center for Laboratories and Research, Albany, New York: AK, HDL-C-DCM
- Pacific Biometrics Research Foundation, Seattle, Washington: AK, HDL-C-DCM

Function of the CRMLN

- CRMLN laboratories participate in bimonthly performance surveys to verify they meet performance criteria
- When CRMLN laboratories meet performance criteria, they are able to certify manufacturers and clinical laboratories.
- Manufacturers and clinical laboratories that meet performance criteria are issued a Certificate of Traceability.
- Certificates are valid for 2 years for manufacturers and 6 months for clinical laboratories.

Reference Methods

- Total Cholesterol**
 - AK method: Saponification of cholesterol with alcoholic potassium hydroxide (KOH), extraction with hexane, evaporation, and development of color with Liebermann-Burchard reagent.
- High Density Lipoprotein Cholesterol**
 - Ultracentrifugation (UC): Separation of very low density lipoprotein (VLDL) by ultracentrifugation before precipitation with heparin manganese (HM) reagent and quantification of cholesterol with the AK method.
 - Designated Comparison Method (DCM): Precipitation with 50K Da dextran sulfate with magnesium chloride and measurement of cholesterol with the AK method.
- Low Density Lipoprotein Cholesterol**
 - Beta-quantification (BC): UC separation followed by quantification of cholesterol in the bottom fraction before and after precipitation with the HM reagent. Quantification of cholesterol is done with the AK method. LDL-C is calculated by the difference between the bottom fraction cholesterol and HDL-C.

Performance Survey Scheme

- Total Cholesterol**
 - 3 unknown pools
 - Each pool analyzed in duplicate in 2 runs
- HDL-C, DCM and UC**
 - 4 unknown pools
 - Each pool analyzed in duplicate in 4 runs
- LDL-C**
 - 4 unknown pools
 - Each pool analyzed in quadruplicate in 4 runs

Statistical Criteria for Performance Surveys

	Trueness criterion	Imprecision criterion
TC	Bias ≤ 1%	CV ≤ 1%
HDL-C	Bias ≤ 1 mg/dL	SD ≤ 1 mg/dL
LDL-C	Bias ≤ 2%	CV ≤ 1.5%

A CRMLN laboratory is notified of unacceptable performance. Consultation with CDC and a special survey are done to reasons performance in these instances.

Comparison Methodology for Certification Programs

- Fresh serum samples are used to compare the test methods to the reference methods
- Samples must meet specific concentration range requirements
- Manufacturers analyze at least 40 fresh samples
- Clinical Labs analyze six fresh samples

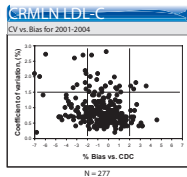
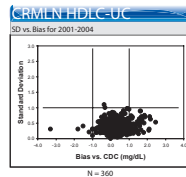
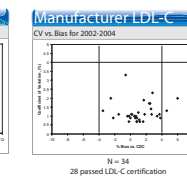
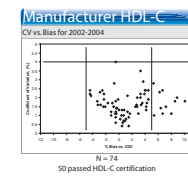
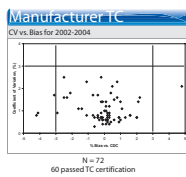
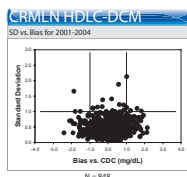
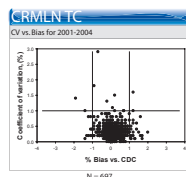
Statistical Criteria used for Certification

Parameter	Criterion
Bias at Medical Decision Points	≤ NCEP goals for inaccuracy
Average % Bias	≤ NCEP goals for inaccuracy
Average Absolute % Bias	≤ NCEP goals for inaccuracy
Among Run CV	≤ NCEP goals for imprecision
CV	≤ 9.975
t test of bias	Not significant at α = 5%
Within-Method Outliers	Manufacturers: 1 allowed Clinical Labs: 0 allowed
Between-Method Outliers	None allowed, but manufacturers may eliminate one sample

NCEP Inaccuracy and Imprecision Criteria for Manufacturer Certification

Parameter	NCEP Inaccuracy	NCEP Imprecision	CRMLN Inaccuracy Allowance*
TC	Bias ≤ 2%	CV ≤ 2%	± 3.3%
HDL-C	Bias ≤ 3%	CV ≤ 4%	-5.5% to 6.5%
LDL-C	Bias ≤ 4%	CV ≤ 4%	-4.8% to 5.1%

* An inaccuracy allowance above the NCEP accuracy limit was applied based on the distribution of the biases obtained from the CRMLN's long term performance.



Conclusions

- CRMLN laboratories maintain traceability to CDC through bimonthly performance surveys. Thus, they are able to provide traceability to manufacturers and clinical laboratories.
- Manufacturers improve calibration when performance criteria are not met.
- The Cholesterol Reference Method Laboratory Network provides a valuable service to the clinical laboratory community through certification programs.
- The CRMLN directly impacts patient care by providing traceability for cholesterol measurements performed in the clinical laboratory setting.

For More Information

- For more information on the Cholesterol Reference Method Laboratory Network, visit <http://www.cdc.gov/labstandards/crmln.htm>
- A list of certified clinical laboratories is available on the CRMLN website.

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