

**Cholesterol Reference Method Laboratory Network
for the
National Reference System for Cholesterol**

***Certification Protocol for
Clinical Laboratories***

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Cholesterol Reference Method Laboratory Network

Clinical Laboratory Certification Protocol

General Information

The National Cholesterol Education Program (NCEP) Adult Treatment Panel has recommended specific medical decision points for serum cholesterol to aid in the detection, evaluation, and treatment of elevated cholesterol levels (1,2,3). These medical decision points were derived from national population studies in which the cholesterol assays were standardized to the Abell-Kendall reference method at the Centers for Disease Control and Prevention (CDC), a major component of the National Reference System for Cholesterol (NRS/CHOL). The NCEP Laboratory Standardization Panel has recommended that clinical laboratories establish traceability of their assays to the NRS/CHOL to properly classify patients according to the NCEP medical decision points (4). CDC has organized the Cholesterol Reference Method Laboratory Network (CRMLN) to provide access to the NRS/CHOL for both manufacturers and clinical laboratories. The CRMLN has developed a method comparison protocol leading to certification for analytical systems.

The NCEP Lipid Standardization Panel recommended that laboratories perform cholesterol analyses with bias $\leq 3.0\%$ from the true value (reference method) and precision, as measured by coefficient of variation (CV), $\leq 3.0\%$. The precision goal can be achieved within each laboratory by adherence to accepted principles of good laboratory practice and quality assurance. The accuracy goal can be achieved through a fresh sample comparison with a CRMLN laboratory.

Two types of systems are available for use in clinical laboratories--homogeneous and heterogeneous. Homogeneous systems are systems in which the instrument, reagent, and calibrator are all manufactured by the one company. Heterogeneous systems are systems in which the components are manufactured by different companies. Clinical laboratories that use a homogeneous system can obtain documentation from the manufacturer of the system's performance and traceability to the NRS/CHOL. However, a manufacturer's demonstration of a product's traceability does not in itself guarantee the accuracy of that product in the hands of every user. Therefore, these clinical laboratories also should participate with a CRMLN laboratory in a split-sample comparison in order to verify the accuracy of their cholesterol measurements. Clinical laboratories that use a heterogeneous system must take primary responsibility for establishing that system's accuracy and traceability to the NRS/CHOL. Because these systems can be unique to a particular laboratory, the CRMLN strongly recommends that these laboratories compare the performance of their system with that of a CRMLN laboratory.

Certification of clinical laboratories is based on a direct comparison of their measurements of fresh patient samples with measurements obtained with the Abell-Kendall reference method.

Certification Process

Universal Precautions **MUST** be observed when collecting and handling any biological material of human origin.

Clinical Laboratory's Preliminary Responsibilities

- ◆ Clinical laboratories must perform the comparison using the analytical system as it is routinely used in the laboratory. The system must be in "good" working condition.
- ◆ Before beginning the split-sample comparison, clinical laboratories should establish that the instrument system meets the precision criteria needed to meet the NCEP guidelines and ensure that the day-to-day precision of the instrument system is $\leq 3\%$. Precision criteria must be met before the split-sample comparison is started. This will save time and money in the long run. Precision testing (such as that outlined in NCCLS Guideline EP5-A, *Evaluation of Precision Performance of Clinical Chemistry Devices* (7)) is recommended.

* Acronyms & Abbreviations: NCEP – National Cholesterol Education Program, CDC – Centers for Disease Control and Prevention, NRS/CHOL – National Reference System for Cholesterol, CRMLN – Cholesterol Reference Method Laboratory Network, CV – coefficient of variation, QC – quality control, HDL – high-density lipoprotein, LDL – low-density lipoprotein.

Clinical Laboratory's Specimen Collection and Handling

Venous serum is the reference blood matrix to be used in all comparisons to establish traceability to the reference method. Venous serum was chosen because it is the matrix used in the epidemiologic studies on which the Adult Treatment Panel of the NCEP based the medical decision points.

Analytical values for all blood matrices other than venous serum must be related to venous serum values through paired sample comparisons. Specimens usually will be serum that has been obtained by venipuncture. However, in some instances, (for example, in certifying a compact analyzer), plasma with either EDTA or heparin, or whole blood collected by venipuncture or finger-stick, is appropriate. Alternate blood matrices include all capillary samples and all anticoagulated samples from both venous and capillary sites. *When a matrix other than venous serum is being evaluated, a paired venous serum sample also must be obtained from each of the six patients used in the comparison.* The clinical laboratory then should analyze the alternate blood matrix samples and submit the serum samples to the CRMLN laboratory for Abell-Kendall analyses. The Certificate of Traceability will indicate the matrix for which the comparison was performed.

A minimum of six fresh patient specimens is required for the comparison. The cholesterol concentration of these samples must be distributed over a clinically meaningful range. To achieve this goal, clinical laboratories must collect two samples in each of the concentration regions listed below.

- Region 1: 100 and 200 mg/dL (2.59-5.17 mmol/L)
Region 2: 200 and 240 mg/dL (5.17-6.21 mmol/L)
Region 3: >240 mg/dL (>6.21 mmol/L)
- ◆ The range of concentration, from the lowest in Region 1 to the highest in Region 3, must be at least 100 mg/dL (2.59 mmol/L).
- ◆ The difference between the concentrations of samples in each of the three regions must be at least 20 mg/dL (0.52 mmol/L).

Difficulty in obtaining a sufficient amount of sample can be overcome by combining two specimens -- **but no more than two**. This combined specimen pool should be treated as an unpooled (single) sample.

A final checklist for collecting appropriate samples is included on the Cholesterol Reference Method Laboratory Network Information Form (see Attachments).

Clinical laboratories should not submit samples that do not meet these guidelines when analyzed on the test system. To meet these sample distribution requirements, the CRMLN recommends that clinical laboratories analyze more than six samples. They then can select six samples from this larger group that meet the sample distribution requirements and send these six samples to the CRMLN laboratory for Abell-Kendall analyses.

The CRMLN laboratory will not analyze the samples by the Abell-Kendall reference method until it has received the results from the clinical laboratory. The CRMLN laboratory will not analyze samples for any clinical laboratory that does not follow these distribution guidelines. If the distribution guidelines are not followed, the clinical laboratory will be asked to send samples that meet these guidelines.

Blood donors, both normal and hypercholesterolemic, must be ambulatory and generally in good health to avoid the potentially interfering effects of unusual lipoproteins, lipemia, or other specimen matrix characteristics that might confound the assessment of accuracy. Liver disease, for example, can lead to abnormal lipoproteins or cause elevated bilirubin levels, both potential sources of interference. Samples with triglyceride levels greater than 300 mg/dL (7.77 mmol/L) or that are noticeably icteric or hemolyzed should not be used. Obtain a volume of each specimen sufficient to analyze it in duplicate by each test method and the reference method. Two milliliters [2.0 mL] of each specimen must be reserved for Abell-Kendall analysis.

Specimen Processing

Follow routine laboratory procedures in processing specimens for serum or plasma collection.

Reference Method Analysis

Transfer 2.0 mL of each serum specimen into a screw-capped cryogenic vial to prevent breakage, leakage, and evaporation. Make certain that the specimen is homogeneous when taking splits of aliquots for analysis by the

reference method. To freeze the samples, place in a nonstyrofoam rack in the freezer; this rack will hold the tube upright in a -20 EC freezer. Insert the tubes directly into the rack. This procedure provides a more uniform rate of freezing. Clearly label the tubes with an identifying code. However, to maintain confidentiality, do not state patients' names or other patient identifiers on the labels.

Test Method Analyses

Keep specimens refrigerated until analysis. To avoid the possibly confounding effects of unstable analyte and matrix interactions, analyze specimens by the test method as soon as possible after collection.

Specimen Analysis

Run specimens in duplicate on 3 separate days--for a total of six analyses per sample--over a period of no more than 5 days (from the date of collection). Operate and calibrate the analytical system according to the usual protocol for the test system.

Information Form

Make copies of the information form for future use.

Complete one form for each analytical system requiring certification. This information will be used to prepare your Certificate of Traceability

Shipment of Specimens for Abell-Kendall Analysis

When the fresh specimens have been tested by each of the various test methods, ship the recorded results for each method to the CRMLN laboratory along with the frozen aliquots for Abell-Kendall analysis.

Contact the laboratory first to arrange for the shipment. Ship the frozen samples on dry ice by overnight express delivery to the CRMLN laboratory. Samples should be shipped between Monday and Thursday to ensure delivery during the workweek. Include results of the analysis of the fresh specimens by the test method and the target values for calibrators used. Before shipping, ensure that the test specimens are clearly and indelibly labeled and that the labels will remain secure during shipment and subsequent storage. Include the Information Form, the Fresh Sample Comparison Result Form, and the Quality Control Results Form with the samples.

A Protocol Checklist is provided. Please refer to the checklist to make sure that all requirements in this protocol have been met.

Follow current federal and state regulations for handling, packaging, and shipping potentially biohazardous materials with regard to containment, labeling, and other procedures.

Observe universal precautions when collecting and handling any biological material of human origin.

Abell-Kendall Analysis, Data Reduction, and Cost

Abell-Kendall analysis will be performed in single on each specimen. The approximate turnaround time for Abell-Kendall analysis and the data analysis is 3-4 weeks from receipt of samples.

Because the Abell-Kendall reference method is manual and tedious and must be maintained within stringent limits for accuracy and precision, the assay is relatively expensive. All of the CRMLN laboratories offer Abell-Kendall cholesterol analyses for the same cost. Please refer to the attached fee schedule for current pricing.

More than one analytical system can be evaluated using one set of fresh specimens. To do this, enough sample must be collected to carry out the entire protocol on all systems and for the Abell-Kendall reference method. The CRMLN Laboratory will charge a data processing fee for each additional system. Several clinical laboratories in a region may share a set of samples and thereby reduce the cost incurred. As with multiple systems from each laboratory, a data processing fee will be charged for additional systems evaluated.

After it meets all the certification criteria, the clinical laboratory will be issued a dated Certificate of Traceability stating that the analytical system (including instrument model, reagent lot, calibrator lot, and matrix) has successfully demonstrated traceability to the NRS/CHOL under the conditions tested. The certificate is valid for 6 months. Clinical laboratories are encouraged to maintain current certification.

The CRMLN publishes a list of clinical laboratories that have been certified through this protocol. The list is available at:

http://www.cdc.gov/labstandards/crmln_members.html

Questions about this protocol should be directed to a CRMLN laboratory (see above). Copies of this protocol are also available at the CRMLN website¹.

¹<http://www.cdc.gov/labstandards/crmln.html>

Statistical Criteria Used For Certification

The criteria used for grading are listed in the following table.

Parameter	Criterion	Statistical Approach
r^2	> 0.975	Linear regression
Bias at 200 mg/dL	≤ 3%	Linear regression equation; NCEP accuracy guideline
Bias at 240 mg/dL	≤ 3%	Linear regression equation; NCEP accuracy guideline
Average % Bias*	≤ 3%	Mathematical mean of biases; NCEP accuracy guideline
Average Absolute % Bias*	≤ 3%	Mathematical mean of absolute biases; NCEP accuracy guideline
Among-run CV	≤ 3%	CV of QC results; NCEP precision guideline
t-test of bias	Not significant at $\alpha = 5\%$	See below
Within-method outliers	None allowed	EP9-A (5), see below
Between-method outliers	None allowed, but may eliminate one sample	EP9-A (5), see below

CRMLN members participate in surveillance to evaluate their performance versus the CDC accuracy base. They are required to meet very strict performance criteria, which are listed in the following table.

Performance Criteria for CRMLN Laboratories

	Accuracy Criterion	Imprecision Criterion
Total Cholesterol	Bias ≤ 1 %	CV ≤ 1 %

The bias of the CRMLN laboratory is the most important factor that needs to be controlled to make appropriate decisions about the performance of test systems (9). Although the CRMLN has achieved a very low bias compared with CDC, some bias still exists. Statistical analysis has been used to study the distribution of the biases obtained by the CRMLN laboratories.

For total cholesterol, results of statistical analysis of the CRMLN survey data collected from December 1995 through February 2000 show a Gaussian distribution. The total number of events (one survey material, analyzed by one lab in one survey) during this time period was 1530. The mean bias was 0.01% with a standard deviation (SD) of the bias of 0.45. The biases, by percentile, were -0.2% for the 25th, 0.0% for the 50th (median), and 0.3% for the 75th. Based on this analysis, we have determined that the CRMLN can allow test systems an additional 0.3% bias above the NCEP accuracy limit. This decision was based on the fact that 50% of the CRMLN bias lies between ± 0.3%.

The following table lists the NCEP performance recommendations, as well as the performance allowances for manufacturers and clinical laboratories, as described above.

Performance Criteria for Manufacturers and Clinical Laboratories

	NCEP Inaccuracy	NCEP Imprecision	CRMLN Inaccuracy Allowance
Total Cholesterol	Bias ≤ 3 %	CV ≤ 3 %	± 3.3 %

The CRMLN has also implemented the use of a t-test to evaluate whether or not the test system's bias is significantly different from the NCEP goal. The variance component of the t-test utilized in the CRMLN programs is the NCEP's maximum allowable imprecision. The rationale for using this value for the variance is that, since the CRMLN's primary goal is to evaluate accuracy, we do not want to penalize a system that has very good precision. The effect of using this t-test is that each test system is evaluated for bias assuming that it has the maximum allowable imprecision. The t-test is performed at various levels for alpha (α). A significant bias at $\alpha=10\%$ should be interpreted as a warning that the bias is very close to the NCEP criterion. A test system that has significant bias at $\alpha=5\%$ is deemed to not meet the NCEP bias criterion and will not pass certification.

The test for within-method outliers is based on the procedure described in NCCLS EP9-A (5). Tests of absolute and relative differences are performed. For the test of absolute differences, the difference between the test method duplicates is calculated for each sample. A test limit is determined which is four times the average difference. Any sample with a difference greater than the test limit is flagged. For the test of relative differences, the difference between duplicates is divided by the test method mean. A test limit is determined which is four times the average relative difference. Any sample with a relative difference greater than the test limit is flagged. Only samples that are flagged by both the absolute and relative tests are within method outliers.

The test for between-method outliers is also based on the procedure described in NCCLS EP9-A (5). Tests of absolute and relative differences between the two methods are performed. For the test of absolute differences, the difference between the test method mean and the reference method mean is calculated. A test limit is determined which is four times the average difference. Any sample with a difference greater than the test limit is flagged. For the test of relative differences, the difference between the test method mean and the reference method mean is divided by the reference method mean. A test limit is determined which is four times the average relative difference. Any sample with a relative difference greater than the test limit is flagged. Only samples that do not pass both tests are between-method outliers.

References

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Cholesterol Reference Method Laboratory Network

Fee Schedule

Total Cholesterol Certification Protocol for Clinical Laboratories

Analysis	Fee
Fresh patient sample comparison 1. 6 patient specimens (duplicate analyses in each of 3 runs on separate days) 2. Single Abell-Kendall analysis by CRMLN Lab 3. Shipping paid by clinical laboratory 4. CRMLN data analysis and Certificate of Traceability	\$375.00
Additional specimens (single Abell-Kendall analyses)	\$62.50/specimen
CRMLN data analysis for additional instruments	\$62.50/method

*Additional administrative charges may apply