National Reference System for Cholesterol

Cholesterol Reference Method Laboratory Network

Total Cholesterol ReCertification Protocol for Manufacturers

October 2004

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General Information

The National Cholesterol Education Program (NCEP)[1] Adult Treatment Panel has recommended specific medical decision points for serum cholesterol to aid in the detection, evaluation, and treatment of people with 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 urged that clinical laboratories standardize their assays to the NRS/CHOL accuracy base to properly classify patients according to the NCEP medical decision points (4). The panel agreed that standardization could be achieved most effectively through the manufacturers of analytical instruments and reagents. To facilitate standardization, CDC 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, reagent applications, calibrators, and reference materials.

The NCEP Laboratory Standardization Panel recommended that laboratories perform cholesterol analyses with bias ≤ 3.0% from the true value (reference method) and precision, as measured by coefficient of variation (CV), ≤ 3.0%. These goals for accuracy and precision suggest that for a single measurement, the allowable total error would be 8.9%. Precision can be improved within each laboratory by adherence to accepted principles of good laboratory practice and quality assurance. Accuracy can be improved by establishment of traceability to the NRS/CHOL through a fresh sample comparison with one of the CRMLN laboratories. ***Although the CRMLN believes that evaluation of total error is important, manufacturers should strive to meet the NCEP's accuracy and precision recommendations separately.***

A major difficulty in standardizing cholesterol methods results from the altered matrix characteristics of calibrators and controls – a result of the manufacturing process – which cause them to react differently from fresh patient specimens in some analytical systems. Calibrating a matrix-sensitive assay system with reference target values of commercially prepared materials results in significant biases when fresh patient specimens are analyzed. Therefore, the only universally reliable approach to establishing appropriate calibration for acceptable accuracy must be based on comparison studies conducted with fresh specimens. This recertification protocol is provided for manufacturers who have successfully completed the CRMLN certification protocol. Recertification is provided for diagnostic systems that have not undergone any instrumental or formulation changes other than lot changes. The recertification protocol for manufacturers is based upon the NCCLS Guideline EP9-A, *Method Comparison and Bias Estimation Using Patient Samples* (5). However, the analytical workload is less arduous than the initial certification process. The procedures are outlined in this document. The protocol includes a comparison with the reference method using 10 fresh specimens. The protocol also includes analysis of a quality control (QC) material in 10 runs. Demonstration of absolute average bias ≤ 3% of the reference method and total CV ≤ 3% qualifies an analytical system for recertification. The setpoints assigned by the manufacturer to that system's calibrators should be appropriate to ensure accurate analytical results on patient specimens in the hands of users.

Because the NCEP recommends that clinical laboratories achieve total error ≤ 8.9% on patient specimens, total error will be calculated. However, certification will be based on meeting the recommended goals for accuracy and precision.

Manufacturers must assume all responsibility for the results and should make the initial contact with the CRMLN laboratory. Manufacturers are advised to contact a CRMLN laboratory before beginning this protocol. A list of CRMLN laboratories is available from the CRMLN Web site[2]. One set of fresh samples can be split and used to evaluate several applications, thus necessitating only one evaluation by the reference method.

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1 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.

2 http://www.cdc.gov/nceh/dls/crmln/memberlabs.htm

The Initial Certification Process

Anyone collecting and handling any biological material of human origin MUST observe Universal Precautions (6).

Purpose

The total cholesterol recertification program is intended to assess the bias of the comparison method against the Abell-Kendall reference method under defined conditions. Not all samples encountered in clinical laboratories are included in the scope of this program. The protocol is designed to verify the calibration of a previously certified analytical system, not the robustness, of the methods evaluated.

Manufacturer's Preliminary Responsibilities

Before pursuing recertification through the CRMLN, manufacturers should establish that their analytical instrument systems meet the following standard specifications:

- Instrument system(s) must be capable of producing discrete number values.
- Instrument system(s) must have had all required preventive maintenance procedures and must be in peak operating condition.
- Precision testing (such as that outlined in NCCLS Guideline EP5-A, *Evaluation of Precision Performance of Clinical Chemistry Devices* (7)) should be done to ensure that total precision is ≤ 3%.

Note: Manufacturers who have the Abell-Kendall reference method set up in house may verify its traceability to the NRS/CHOL by certification through the CRMLN. Although this internal verification does not substitute for certification of the manufacturer's products, manufacturers can use a certified Abell-Kendall method in house to check their products before evaluation of traceability by a CRMLN laboratory.

Manufacturer's Specimen Collection

Specimens should be fresh; fasting donors are preferred. Use of frozen specimens is *not* recommended unless the manufacturer has conducted a careful freeze/thaw study and determined that use of frozen specimens in the test system would not compromise the results of this traceability study. (The CRMLN has developed a Sample Stability Protocol that can be used to determine the stability of frozen samples compared to fresh samples. This protocol is available from the CRMLN Web site[3].) The recertification protocol is designed to evaluate analytical method bias only. Therefore, variation from preanalytical sources must be eliminated or minimized. Manufacturers should carefully follow the protocol for sample collection and processing described in this section.

The recommended sample matrix is serum; however, the comparison should be performed using the sample matrix for which the analytical system is designed. Venous serum is the matrix to be used for all comparisons designed to establish traceability to the accuracy base. Values for all other blood matrices must be traced to venous serum values through paired sample comparisons. Alternate blood matrices would include all capillary samples (including serum) and all anticoagulated samples from both venous and capillary sites. For example, if a manufacturer's system is designed to analyze capillary plasma and the manufacturer wishes to be certified for this matrix, the manufacturer should collect paired venous serum and capillary plasma samples from the patients used in the comparison. The manufacturer should then analyze the capillary plasma samples and submit the venous serum samples to the CRMLN laboratory for analysis.

Collect and analyze 10 fresh specimens from patients. The cholesterol concentration levels of these specimens should be distributed over a clinically meaningful range, using the following distribution:

- 2 samples from 120 to 180 mg/dL (3.10 to 4.67 mmol/L)
- 3 samples from 181 to 220 mg/dL (4.68 to 5.71 mmol/L)
- 3 samples from 221 to 260 mg/dL (5.72 to 6.74 mmol/L)
- 2 samples from 261 to 400 mg/dL (6.75 to 10.34 mmol/L)

3 http://www.cdc.gov/nceh/dls/crmln/pdfs/FrozVsFreshProtocolOct04.pdf

A worksheet for specimen distribution is attached as an aid.

These specimens must:

- be free of interfering substances known to affect the system being tested (e.g., hemolysis, icterus, marked lipemia[4]);
- not include samples that the product's package insert indicates should be excluded;
- be collected in sufficient quantity (see sample volume requirements for Abell-Kendall testing below); and
- be collected using good laboratory practice (such as outlined in NCCLS Guideline H3 A4, *Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture* (8)).

To make the certification process reflect reality, the protocol followed should mimic, as much as is possible, the conditions present in the clinical laboratory. Manufacturers should develop package inserts that include information about time frames for storage and analysis of patient samples. Storage times and methods should be evaluated as part of the method development.

Balancing the need for reflecting conditions in clinical laboratories is the need to have the samples analyzed by the manufacturer be as similar as possible to those analyzed by the CRMLN laboratory. Serum should be separated from red cells within 2 hours of collection. The serum for the comparison may be stored up to 2 days at 4°C after separation.

The minimum amount of serum needed per sample for Abell-Kendall analysis is 1.5 mL. Samples intended for Abell-Kendall analysis should be dispensed into 2 mL cryogenic vials, frozen (at -70 °C or lower) at the same time as analysis by the test method begins, and stored frozen until all runs are complete. Specimens need to be clearly identified by coded identification numbers and **no patient identifiers**. Specimens must be securely contained in cryogenic vials to prevent breakage, leakage, and evaporation.

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. The combined specimen pool should be mixed well before aliquoting.

Some of the same samples used for recertification of other analytes (HDL cholesterol, LDL cholesterol, and triglyceride) by the CRMLN may be used. Because all samples are unlikely to meet the sample distribution guidelines for all analytes, additional samples should be collected. This will ensure that all analytes meet the guidelines.

Collection of patient samples is the responsibility of the manufacturer. However, the CRMLN laboratory may assist in this process by collecting some or all of the specimens, as long as the specimens analyzed by the test method are fresh and can be analyzed within 2 days. Manufacturers can work with a local clinic or hospital to collect samples.

Manufacturer's Quality Control

To evaluate total error, estimates of both inaccuracy and imprecision are needed. The inaccuracy can be obtained from the split sample comparison with the reference method. However, to estimate imprecision, the CRMLN requires that manufacturers provide QC data obtained from 10 separate runs. The recommended concentration range for the QC material is 200 to 240 mg/dL (5.2 to 6.2 mmol/L). A frozen human serum pool is more representative of fresh patient samples and is preferable to a processed (e.g., lyophilized) material. The latter may result in higher imprecision leading to a falsely high total error. The QC material must be analyzed in single using the complete analytical system being evaluated. The runs must include those used in the split sample comparison described below. Use the attached Quality Control Results Form for reporting the results.

Manufacturer's Specimen Analysis

The CRMLN recommends that manufacturers perform the comparison analyses at the manufacturing site; however, manufacturers may have a clinical laboratory using their system perform the split comparison with the

4 Lipemia is defined as triglyceride levels >300 mg/dL.

reference method. Clinical laboratories that perform successful comparisons on behalf of a manufacturer will also receive a Certificate of Traceability for Total Cholesterol.

Calibration is the key to achieving accuracy; therefore, these comparison runs should represent the conditions recommended to customers.

More than one system may be recertified using one set of samples. **If more than one lot of reagents or calibrators are to be recertified, all of the fresh samples should be analyzed with each lot.** Each combination of instrument, reagents, and calibrator that meets the NCEP performance recommendations will be issued a Certificate of Traceability for Total Cholesterol.

- Follow the protocol for collecting, dispensing, and storing samples as described on pages 3 - 4 of this document. Store the samples at 4˚C before analysis and begin analysis of all specimens within 2 days of collection. Analyze each sample by the test method in duplicate for total cholesterol.
- Include the QC material selected above in every analytical run.
- Analyze the specimens over 10 runs, one sample per run. More than one run per day is acceptable, provided that the runs are separated by 2 hours and the analytical system is recalibrated for each run (NCCLS definition of a run, per EP5-A). Manufacturers of point-of-care instruments that are factory-calibrated should define their "runs" similarly, i.e. by waiting two hours between analysis of pairs of samples. Manufacturers may also choose to use more than one instrument of the same model to accomplish the runs in a shorter period of time. However, all of the samples analyzed in the comparison must originate from 10 unique donors.
- If more than 10 samples are to be analyzed, divide the excess samples evenly among the 10 analytical runs.
- Run days need not be consecutive, but all testing should be completed within a reasonable time period (i.e., less than 1 month).
- Perform testing following the instructions provided in the package labeling (i.e., do not follow in-house modifications).
- If an instrument problem develops during a run or internal QC is unacceptable, retest the specimens from that run after the problem has been identified and corrected.
- Complete the Information Form, the Fresh Sample Comparison Result Form, and the Quality Control Results Form. Photocopy the blank forms and save them for future comparisons.

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. The CRMLN laboratory cannot analyze the samples until the method results have been received.

Contact the CRMLN 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.

Abell-Kendall Analysis, Data Reduction, and Cost

The CRMLN laboratory will perform Abell-Kendall analyses in duplicate on each specimen over a minimum of 3 runs and will provide manufacturers with all results and statistical analysis. The approximate turnaround time for Abell-Kendall analysis and data analysis is 3-4 weeks from receipt of samples but could take longer depending on the analytical workload at the CRMLN laboratory. If a manufacturer wishes to evaluate more than one analyte (e.g., total cholesterol, HDL cholesterol, and LDL cholesterol), the CRMLN laboratory analyses could take longer than 3-4 weeks.

Because the Abell-Kendall reference method is manual and tedious and must be maintained within stringent limits for accuracy and precision, the assay is costly. All of the CRMLN laboratories offer Abell-Kendall cholesterol analyses for the same cost. Refer to the attached fee schedule for current pricing. Manufacturers may test the same set of specimens on several instrument, calibrator, or reagent systems. A nominal fee will be charged for certifying each additional system to cover data analysis costs.

The data analysis program used by the CRMLN will select the first replicate from each specimen from each run to use to evaluate the bias. The data from all 10 samples and replicates will be used to evaluate for outliers.

After meeting all the recertification criteria, the manufacturer will be issued a dated Certificate of Traceability for Total Cholesterol, stating that the analytical system (including instrument model, reagent lot, and calibrator lot) has successfully demonstrated traceability to the NRS/CHOL under the conditions tested. The Certificate will list the bias versus the reference method, the total CV, and the calculated total error. A separate certificate will be issued for each analytical system that successfully meets the NCEP performance recommendations. The date used on the certificate as the “Date of Comparison” is the date that the data is analyzed at the CRMLN laboratory. Certificates expire 2 years after this date. Manufacturers are encouraged to maintain current certification.

Once the accuracy of an analytical system has been validated, conventional in-house QC procedures should be adequate to monitor the system. However, if shifts occur, the manufacturer should undertake another direct comparison with the Abell-Kendall method in a CRMLN laboratory to reset the system for optimal accuracy. Changes in lots of calibrators or reagents should be carefully checked to maintain accuracy. If the reagent or calibrator formulations or the instrumentation are substantially modified, a new direct comparison will be needed to verify accuracy under the new conditions. This Certification Protocol should be followed in this case.

The CRMLN publishes a list of manufacturers' systems that have been certified through this protocol. The list is available at on the CRMLN Web site[5] and includes all systems with a current Certificate of Traceability for Total Cholesterol.

Proficiency surveys, such as those offered by the College of American Pathologists, are essential in providing feedback on the performance of analytical systems. Values differ with certain systems using lyophilized survey pools. Manufacturers should assess the validity of these lyophilized survey pools on their systems and advise users of any offset or bias. Eventually any interim confusion should be reduced, and better setpoints, survey pools, and control materials will allow for better reliability in maintaining accuracy.

Questions about this protocol should be directed to a CRMLN laboratory (see the CRML Web site[6] for a list of CRMLN laboratories.) Copies of this protocol are also available at the CRMLN website[7].

5 http://www.cdc.gov/nceh/dls/crmln/totaltable.htm
6 http://www.cdc.gov/nceh/dls/crmln/memberlabs.htm
7 http://www.cdc.gov/nceh/dls/crmln/crmln.htm

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
Z-test of bias	Not significant at α = 5%	See below
Within-method outliers	1 allowed	EP9-A (5), see below
Between-method outliers	None allowed, but may eliminate one sample	EP9-A (5), see below

*The regression and bias parameters will be evaluated using 10 samples, analyzed in single; one sample from each analytical run.

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 Z-test to evaluate whether or not the test system's bias is significantly different from the NCEP goal. The variance component of the Z-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 Z-test is that each test system is evaluated for bias assuming that it has the maximum allowable imprecision. The Z-test is performed at various levels for alpha (α). A significant bias at α = 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 $\alpha \leq 5\%$ 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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2. National Cholesterol Education Program. Report of the National Cholesterol Education Program Expert Panel on detection, evaluation, and treatment of high blood cholesterol in adults. Arch Intern Med 1988;148:36–9.

3. National Cholesterol Education Program (http://www.nhlbi.nih.gov/guidelines/cholesterol/). Executive summary of the Third Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATP 111). JAMA 2001; 285; 2486-96.

4. Recommendations for improving cholesterol measurement: a report from the Laboratory Standardization Panel of the National Cholesterol Education Program. (NIH Publication No. 90-2964). Bethesda, MD: National Institutes of Health, February 1990.

5. NCCLS. Method comparison and bias estimation using patient samples; approved guideline. NCCLS document EP9-A. Wayne, PA: NCCLS, 1995. Copies may be purchased by calling NCCLS at (610) 688-0100 or from the NCCLS Web site at http://www.nccls.org

6. Perspectives in Disease Prevention and Health Promotion Update: Universal Precautions for Prevention of Transmission of Human Immunodeficiency Virus, Hepatitis B Virus, and Other Bloodborne Pathogens in Health-Care Settings. MMWR June 24, 1988 / 37(24);377-388. http://www.cdc.gov/mmwr/preview/mmwrhtml/00000039.htm.

7. NCCLS. Evaluation of precision performance of clinical chemistry devices; approved guideline. NCCLS document EP5-A. Wayne, PA: NCCLS, 1999. Copies may be purchased by calling NCCLS at (610) 688-0100 or from the NCCLS Web site at http://www.nccls.org.

8. NCCLS. Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard — Fourth Edition. NCCLS document H3-A4. Wayne, PA: NCCLS, 1998. Copies may be purchased by calling NCCLS at (610) 688-0100 or from the NCCLS Web site at http://www.nccls.org.

9. Bennett ST, Eckfeldt JH, Belcher JD, Connelly DP. Certification of cholesterol measurements by the National Reference Method Laboratory Network with routine clinical specimens: Effects of network laboratory bias and imprecision. Clin Chem 1992;38:651-7.

Cholesterol Reference Method Laboratory Network

Fee Schedule

Total Cholesterol ReCertification Protocol for Manufacturers (using Abell-Kendall reference method for cholesterol)

Manufacturers	
Fresh Sample Comparison 1. Duplicate Abell-Kendall analysis of 10 patient specimens 2. Shipping charges paid by manufacturer 3. Data analysis for one instrument/method, material, or reagent application 4. One repeat data analysis, if necessary	$3,200.00
Additional specimens (duplicate Abell-Kendall analyses)	$100.00/specimen
Data analysis for additional applications	$100.00/method

Cholesterol Reference Method Laboratory Network

Information Form

The following information form should be completed carefully and accurately. This information will be used to prepare your Certificate of Traceability.

- Please photocopy this blank form and retain it for future submissions.
- Please prepare a copy of your data and retain it for laboratory records.

For registered products, please indicate preferred designation: Registered Trademark ® or Trademark ™.

Laboratory Name			
Laboratory Address			
Contact Name		Phone	
Email Address		Fax	
Send Bill To (If different from above.)			
PO Number			
Date Specimens Sent		Date Specimens Received	

Analyte		Method	
Instrument		**Calibrator**	
Manufacturer		Manufacturer	
Trade Name		Trade Name	
Model Number		Lot Number(s)	
Reagent		Calibrator Set Point(s)	
Manufacturer		**Matrix/Sample Type**	
Trade Name		Anticoagulant (if applicable)	
Lot Number(s)		Concentration	

Comparision Date

CRMLN Laboratory: Complete this section and send the form to Mahnaz Dasti at CDC.
Fax: (770) 488-4192, Email: mdasti@cdc.gov

CRMLN Laboratory Name					
Date of Data Analysis		Date Report Received		Date Certificate Sent	
Director's Signature		Check One:	Passed		Failed

Cholesterol Reference Method Laboratory Network

Total Cholesterol
Fresh Sample Comparison Results Form

Please photocopy this blank form and retain it for future comparisons.

Run #1	**Date**	
ID Number	Result #1	Result #2
Run #3	**Date**	
ID Number	Result #1	Result #2
Run #5	**Date**	
ID Number	Result #1	Result #2
Run #7	**Date**	
ID Number	Result #1	Result #2
Run #9	**Date**	
ID Number	Result #1	Result #2

Run #2	**Date**	
ID Number	Result #1	Result #2
Run #4	**Date**	
ID Number	Result #1	Result #2
Run #6	**Date**	
ID Number	Result #1	Result #2
Run #8	**Date**	
ID Number	Result #1	Result #2
Run #10	**Date**	
ID Number	Result #1	Result #2

Questions about this protocol should be directed to the CRMLN Laboratory.

Cholesterol Reference Method Laboratory Network

Total Cholesterol
Specimen Distribution Form

The following chart is supplied to assist you (or the off-site laboratory that supplies you with serums) in selecting specimens that will adequately cover the concentration ranges recommended by the EP9-T protocol. *[This form is provided as an aid; it is not necessary to return it to the CRMLN laboratory.]*

Please photocopy this blank form and retain it for future comparisons.

Total Cholesterol Specimen Distribution

Concentration (mg/dL)	120-180	181-220	221-260	261-400
Number Needed	(2)	(3)	(3)	(2)
	1.	1.	1.	1.
	2.	2.	2.	2.
		3.	3.	

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Quality Control Results Form for Total Cholesterol

Report single analyses of any quality control material with a total cholesterol concentration of 200 – 240 mg/dL (recommended). Data must be obtained with the analytical system under evaluation and must include the runs used in the split sample comparison.

Please photocopy this blank form and retain it for future comparisons.

Run Number	Date	Result
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		

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Protocol Checklist

Yes	No	
○	●	Have you collected a minimum of 10 samples?
○	●	Were the sample concentrations distributed according to the guidelines found in the protocol?
○	●	Were all of the fresh samples analyzed in duplicate?
○	●	Was one fresh sample analyzed in each of 10 analytical runs?
○	●	Were all runs separated by at least two hours?
○	●	Were the same instrument, reagent lot, and calibrator lot used in ALL analytical runs of the fresh samples?
○	●	Have you submitted data for 10 analytical runs of a quality control material?
○	●	Do the 10 analytical runs of the quality control material include the analytical runs of the fresh samples?
○	●	Have you completed the Information Form provided in the protocol?
○	●	Have you completed the Fresh Sample Comparison Results Form provided in the protocol?
○	●	Have you completed the Quality Control Results Form provided in the protocol?
○	●	Does your CV for the QC data meet the NCEP goal of ≤ 3%?
○	●	Have you provided sufficient volume of serum for the CRMLN laboratory, as described in the protocol?
○	●	Have you notified the CRMLN laboratory of your plans to ship samples?

Have you checked “Yes” for each item? If not, please make sure that you have met all of these requirements before sending samples and data to the CRMLN laboratory. We are not able to analyze samples or data that do not meet these requirements.