CHOLESTEROL REFERENCE METHOD LABORATORY NETWORK (CRMLN)

TOTAL GLYCERIDES CERTIFICATION PROTOCOL

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GENERAL INFORMATION

Accurate and reliable measurements of triglycerides (TG) are critical for correctly assessing a patient's risk for developing cardiovascular diseases (CVD), and for monitoring patients being treated for unhealthy lipids levels. TG measurements are part of the lipid panel along with total cholesterol, HDL cholesterol, and LDL cholesterol used to assess a person's risk for developing CVD. TG measurement results can also be used to identify individuals with very high TG levels, for whom some HDL-cholesterols or LDL cholesterol assays may not provide accurate measurement results.

The Centers for Disease Control and Prevention (CDC) program for standardization of blood lipids together with the Cholesterol Reference Method Laboratory Network (CRMLN) aims at ensuring that blood lipid measurements, such as TG measurements, have an analytical accuracy and precision within the analytical performance limits defined by the clinical and public health communities. The National Cholesterol Education Program (NCEP) developed analytical performance criteria for blood lipids and recommended for total glycerides a maximum allowable mean bias of ±5.0% from the reference value, a maximum allowable mean precision of <5.0% expressed as coefficient of variation (CV, %) based on a desired total error of ±15.0%.[3,4,5,6,7] The CDC program for standardization of blood lipids adopted these criteria when evaluating and certifying TG assays. TG assays, in this context, can include complete analytical systems, and/or key reagents and calibrators used with specified analytical systems. A CDC standardized and certified assay demonstrated through a thorough assessment a performance that is within the NCEP recommended criteria and traceable to SI through the CDC reference system.

The Cholesterol Reference Method Laboratory Network (CRMLN) is a network of highly specialized laboratories operating reference methods for blood lipids with high analytical precision and accuracy. The high quality analytical performance of CRMLN laboratories is ensured through regular assessments by the CDC standardization program for blood lipids, which compares measurement results of CRMLN participants to those of the CDC Lipids Reference Laboratory. For further details about CRMLN and the CDC Reference Laboratory see https://www.cdc.gov/labstandards/crmln.html.

CDC established CRMLN to ensure sufficient and appropriate reference laboratory capacity is available to assay manufacturers and laboratories worldwide. Furthermore, by working with CRMLN member laboratories, assay manufacturers and laboratories can ensure that their assays are assessed in an appropriate and consistent manner, and are traceable according to ISO 17511.

CRMLN laboratories work with assay manufacturers and laboratories on performing a method comparison study that includes information on analytical precision. The data are then provided to the CDC standardization program for the assessment of analytical accuracy and precision of TG assays. Assays meeting the NCEP performance criteria are considered standardized by CDC and are listed on CDC website (https://https://www.cdc.gov/labstandards/crmln_certified_manufacturers.html).

SCOPE

This protocol describes the prerequisites and procedures for assessing the analytical accuracy and precision of assays intended for measuring TG in patient care, clinical research and public health.

The analytical performance is assessed within the reportable range of the assay and using serum samples with concentrations that are typically observed in patient care and public health.

The protocol can be applied to established assays already used in patient care, research and public health, as well as to assays that are in development and not yet finalized for use in patient care and public health.

While the data obtained with this protocol can provide information on potential sources of measurement bias and imprecision, the determination of such sources is outside the scope of this protocol. Also, this protocol is not intended to assess the diagnostic performance of TG assays.

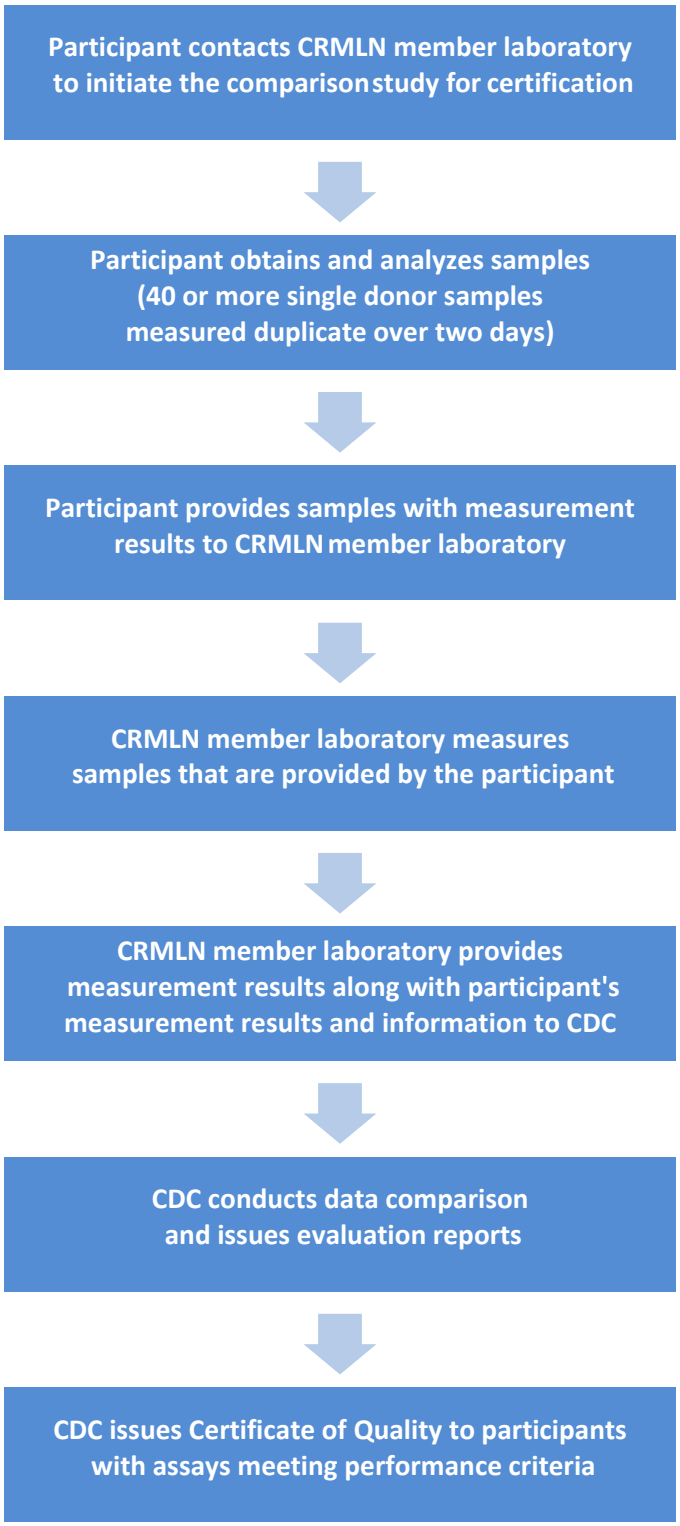
The primary aim of this protocol is to verify calibration accuracy and to provide information on sources that could lead to potential inaccurate measurements. This protocol uses the mean bias as parameter reflecting the calibration bias. Furthermore, this protocol aims at assessing measurement precision observed during regular operation of the assay.

The participant assumes responsibility for all data and results provided to the CMLN Laboratory and CDC. The procedure follows the well-established Clinical and Laboratory Standards Institute Guideline "EP09

Measurement Procedure Comparison and Bias Estimation Using Patient Samples"[8]. In brief, single donor patient samples are measured for TG by the assay manufacturer or laboratory (called "participant" in this protocol) and by the CRMLN member laboratory. Measurement accuracy is determined by comparing the reference target value to the value reported by the participant. Additional data are collected from the participant to assess measurement precision. Measurement accuracy and precision are then compared to the NCEP analytical performance criteria for TG. Participants meeting the NCEP criteria are certified by the CDC program. Certified participants are listed on CDC's website: https://www.cdc.gov/labstandards/crmln_certified_manufacturers.html. Certificates are valid for two years.

CERTIFICATION PROCESS

Overview



Safety Consideration

Any personnel collecting and handling any biological material of human origin MUST observe Universal Precautions[9]. Relevant regulations and policies need to be followed when shipping and handling biological samples.

Initiation of the certification process

Before beginning the actual comparison study for certification, the participant needs to contact a CRMLN member laboratory to allow for scheduling CRMLN member laboratory measurements and to obtain further information such as sample volume requirements from the CRMLN member laboratory.

A list of CRMLN member laboratories is available from the CRMLN website at https://www.cdc.gov/labstandards/crmln_members.html. If a CRMLN member Laboratory cannot be identified, contact the CDC Clinical Standardization Programs for Lipids at cdclsp@cdc.gov for assistance.

Requirements for participants

Before pursuing certification, participant should establish that their analytical instrument systems meet, at a minimum, the following standard specifications.

- Instrument system(s) must be capable of producing discrete number values.
- Precision testing at three different concentration levels performed over 20 independent analytical runs (i.e., as outlined in CLSI Guideline EP05-A3, *Evaluation of Precision Performance of Clinical Chemistry Devices)*[10] is completed and data can be made available to the CRMLN member laboratory and CDC Clinical Standardization Programs
- Instrument system(s) must have had all required preventive maintenance procedures and must be operational as intended by the participant.

The participant is responsible for obtaining and providing to the CRMLN member laboratory the appropriate number of samples with the samples characteristics described in this section.

- The recommended sample for use with this protocol is serum. Please contact the CRMLN member laboratory, if other samples are intended to be used.
- Obtain 40 or more single-donor samples. Prepare paired aliquots for participant's and CRMLN member laboratory's analyses. Contact CRMLN member for specimen volume requirements.
- Clinical decision levels are often based on data obtained with serum. If other blood matrices are used for method comparison, then values need to be traced to venous serum values through paired sample comparisons. Data from this comparison need to be made available to the CRMLN member and CDC.
- All samples need to be collected, handled and shipped in a manner that preserves the integrity of the sample.
- The Clinical and Laboratory Standards Institute (CLSI) is one organization that provides guidance documents for sample collection and handling (i.e., CLSI H03-A5, *Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture*[11]).

The samples used for this program need to have the following sample characteristics:

- Samples can be fresh or frozen; from fasting or non-fasting donors.
- The total glycerides concentration levels of these samples should be distributed over a clinically relevant range and be within the analytical measurement range of the analytical system.
- It is recommended to have a distribution as close as possible to the following target distribution (out of 40 or more samples):
  - 25% samples ≤ 149.99 mg/dL (≤ 1.684 mmol/L)
  - 25% samples from 150.00-199.99 mg/dL (1.695 - 2.249 mmol/L)
  - 25% samples from 200.00-399.99 mg/dL (2.260 - 5.639mmol/L)
  - 25% samples from ≥ 400.00 mg/dL (≥ 5.650 mmol/L)

Do not include samples that are not intended to be measured with the assay (i.e., as described in the product's package insert)

Samples need to be provided to the CRMLN member laboratory in sufficient quantity (contact CRMLN member laboratory for volume requirements)

The samples should not be altered or modified (i.e., through spiking or stripping)

Samples need to be clearly identified by coded identification numbers and should not contain any donor identifiers. The samples must be securely contained in cryogenic vials to prevent breakage, leakage, and evaporation.

40 or more single-donor samples from different donors should be used. It is recommended that more than 40 samples be collected, measured and sent to the CRMLN member laboratory to ensure sufficient number of samples are present to cover target distribution after outliers are removed.

One set of samples can be used for different certification procedures (i.e., certification for TG and total cholesterol), if the sample requirements outlined in the specific protocols are fulfilled.

The participant needs to provide quality control (QC) data. QC samples needs to be measured along with samples that are used in certification. The concentrations of the QCs should be within analytical measurement range of the assay. The QC samples should be analyzed together with single-donor samples used for this comparison study.

Comparison Study

Measurements at the participant's laboratory

We understand that participants perform the analyses of the samples at their sites (i.e., manufacturing site). All analyses should be performed on the same instrument model using the same lot of reagents and calibrators.

- Analyze 40 or more samples in duplicate over two separate days.
- Include the QC material selected in every analytical run as stated in section (c)(ii)(1).
- Manufacturers of point-of-care instruments that are factory-calibrated should define their "runs" similar to other type of assays.

- 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 40 unique donors.
- 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 samples from that run after the problem has been identified and corrected.
- Data is reported to CRMLN member laboratory using the provided CDC data submission template. Same samples that are used are to be shipped to CRMLN member laboratory for value assignment as shown in section 2).

Shipment of samples to CRMLN Member Laboratory

- Ship the paired frozen samples on dry ice to the CRMLN member laboratory. Shipments needs to be scheduled with the CRMLN member laboratory.
- Samples need to be clearly and indelibly labeled and that the labels remain secure during shipment and subsequent storage.
- Samples need to be shipped according to relevant policies and regulations. Contact the CRMLN member laboratory for specific policies and regulations.
- Assay information, results and QC results are to be provided in CRMLN data submission template at the time of shipment.
- Samples need to be provided along with filled data submission template.

Measurement at the CRMLN member laboratory

- The CRMLN member laboratory performs analyses using reference method in duplicate on each sample over a minimum of three runs.
- The CRMLN member laboratory conducts preliminary data evaluation and provide the results to CDC.

Data evaluation at CDC

CDC will perform data analysis and evaluation based on CLSI EP09-A2.[8]

- CDC evaluates the integrity of data and information provided
- The data from all 40+ samples and replicates are used to evaluate for outliers
- Analytical performance criteria mentioned in Appendix A are used to determine if the participant's assay is certified.
- CDC issues evaluation report and provides to the CRMLN member laboratory and participant.

Certification

Participants meeting all analytical performance criteria in Appendix A are issued Certificates of Quality by CDC stating that the analytical system (including instrument model, reagent lot, and calibrator lot) has successfully demonstrated accuracy, precision and total error and thus

traceability to the conditions tested.

The certification date used on the certificate is date of data comparison by CRMLN member laboratory. Certificates expire two years after this date. Participants are encouraged to repeat certification process every two years to maintain certification status.

Post certification

The analytical performance of an analytical system can change over time. It is recommended to monitor the analytical performance over time to ensure changes are detected in time. The CDC Lipid Standardization Program (CDC LSP) or equivalent programs can be used to monitor the analytical performance over time.

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APPENDIX A: ANALYTICAL PERFORMANCE CRITERIA USED FOR CERTIFICATION

Table 1: Analytical Performance Criteria Used for Certification

Parameter	Criterion	Statistical Approach
Mean % Bias	±5.0%	NCEP[7]
Mean % CV (Coefficient of Variance)	≤5.0%	NCEP[7]
Total error	±13.3%	Each measurement must meet total error criteria. Total error (TE) criterion is calculated using formula where TE = Bias Criteria + 1.65 x Imprecision Criteria, where factor of 1.65 implies that 95% of the results will fall within the total error limit, given a gaussian distribution.[12]