VITAMIN D STANDARDIZATION CERTIFICATION PROGRAM

STANDARDIZATION OF TOTAL SERUM 25-HYDROXYVITAMIN D MEASUREMENTS

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GOALS

The objective of the Centers for Disease Control and Prevention's Vitamin D Standardization-Certification Program is to improve the diagnosis, treatment, and prevention of diseases and disorders by assuring accurate and reliable clinical vitamin D measurements.

PRINCIPLE

Standardization of total 25-hydroxyvitamin D [25(OH)D] measurements in serum, defined as the sum of 25-hydroxyvitamin D2 [$25(OH)D_2$] and 25-hydroxyvitamin D3 [$25(OH)D_3$], will be established through method comparison and bias estimation between a reference laboratory and the testing laboratory. The measurements from the reference laboratory are comparable to reference measurements performed at the National Institute for Standards and Technology and the University of Ghent and meet analytical performance criteria suggested in the literature.[1] Single-unit, fresh-frozen serum samples will be used, and the observed bias will be compared to predefined limits. A laboratory is considered standardized when the observed bias is within the predefined limits.

PROTOCOL

Materials

The materials used for method comparison and bias estimation are non-pooled sera from single donors obtained following the protocol from the Clinical and Laboratory Standards Institute (CLSI) C37-A.[2] Sera prepared according to this protocol have shown to be commutable and were recommended for use in trueness control and calibration studies.[3] The materials underwent two freeze-thaw cycles and are approximately within the range of 25(OH)D commonly observed in most populations.

Procedure

The standardization protocol consists of two phases:

In Phase 1, participants will receive 40 single-donor human serum samples with openly indicated concentrations assigned by the reference laboratory. The participants may use these 40 samples to perform a bias assessment and adjust their calibration as needed prior to starting Phase 2. If requested, CDC will provide assistance on technical aspects of the measurement process and assist with calibration adjustments. This phase is optional for laboratories that have already completed comparisons to the reference laboratory and are satisfied with their performance. If needed, participants can request additional Phase 1 samples during enrollment.

In Phase 2, participants will receive four quarterly shipments of 10 single donor human serum samples in each shipment (two vials per sample, for a total of 20 cryovials per shipment) with blinded concentrations. Samples are to be analyzed on two different days in duplicate, for a total of 40 measurements.

All participants are responsible for fulfilling internal quality control requirements. Rejected runs need to be repeated.

All shipments will be made the first full weeks in **February**, **May**, **August**, and **November** for both Phase 1 and Phase 2 materials.

Data Submission

All data must be submitted using the Excel data submission template provided to Standardization@cdc.gov.

Data for each quarterly challenge are to be submitted to CDC within **four weeks** of sample receipt to allow for data analysis and feedback prior to the next quarterly shipment. Individual measurement results are to be reported to three significant figures and in nmol/L units. At this time only total 25(OH)D will be evaluted. Assays measuring $25(OH)D_2$ and $25(OH)D_3$ separately can report these analytes in addition to total 25(OH)D. Additional information about calibrators, reagents, and other measurement parameter will be requested in the data template.

Reference Values

Reference values are assigned to the sera by the reference laboratory, which uses ID-HPLC/MS/MS and certified primary standards from the National Institute for Standards and Technology. The method is recognized by the Joint Committee for Traceability in Laboratory Medicine (JCTLM) as a reference measurement procedure (RMPs) of a higher-order. Therefore, these samples are traceable as described in ISO 17511.[4]

DATA ANALYSIS

Feedback from each quarterly challenge will be provided 4 weeks prior to the shipment of the next challenge.

At the end of the year, a final assessment is performed using data from all four quarters. Bias and imprecision of the measurement procedure will be assessed. Results and conclusions from method comparison and bias estimation will be communicated to the participating laboratory in writing by CDC. Method comparison and bias estimation will be performed by the procedure described in CLSI document EP9-A2 "Method Comparison and Bias Estimation Using Patient Samples."[5] The acceptable bias, imprecision, and total error are $\pm$5.0%, ≤10.0%, and ±21.5% respectively, based on the model of biological variability.[1] At this time, only mean percent bias and imprecision will be used for certification.

The certificate is valid for one year and needs to be renewed on an annual basis, with successful participation in Phase 2 in subsequent years. CDC can provide technical assistance to help resolve any problems in meeting the performance standards, thus helping to insure the participant's long-term success in maintaining standardized 25(OH)D measurements.

CDC will issue annual certificates documenting enrollment and performance in the CDC program to all participants that meet the established criteria. Participation will remain anonymous, and with participants' approval, laboratories passing the predefined limits will be listed on the CDC Website and may be mentioned in presentations given by CDC staff.

Data submitted by participants will be retained at the CDC following current CDC rules and policies.

COLLABORATION FEES

Participation is voluntary. The fees associated with this process (samples, data processing, and reporting) will be covered by the participant. Shipment costs will also be covered by the participant.

Collaboration fees based on type of enrollment:

Option A: Phase 1 and Phase 2
Enrollment **$9,000** (+administration fees)
Initial 40-sample shipment and four challenge shipments over 12 months, including data processing and reporting

Option B: Phase 2 Only
Enrollment **$6,000** (+administration fees)
Four challenge shipments over 12 months, including data processing and reporting

Option C: Phase 1 Only Samples
Enrollment **$3,000** (+administration fees)
Initial 40-sample shipment (does not include enrollment in the certification program)

Please note that CDC does not handle any payments directly. Instead, collaboration fees will be coordinated by and made to the CDC Foundation.

CDC Foundation Contact
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LOGISTICS

Upon receipt of payment, samples will be shipped frozen with FedEx Priority Overnight on dry ice. All shipments will be made during the first full week of the following months: **February**, **May**, **August**, and **November**. Shipping address, FedEx account number, and contact person must be provided by the participant.

Phase 1 shipments will be made upon receipt of payment.
Phase 2 shipments will be made three months after Phase 1 samples obtained or when participants are ready. Shipments will be repeated quarterly for 12 months.

Upon receipt, samples should be immediately stored frozen (at-20^{0}C or lower) until use. Participants should assess the shipment for completeness, for samples that are damaged or

leaking, and whether samples arrive frozen. Participant must send an e-mail within 24 hours to confirm sample receipt and to report any discrepancies or problems (Standardization@cdc.gov).

SAFETY

All materials need to be considered potentially infectious. Observe universal precautions.

REFERENCES

1. Stöckl D, Sluss PM, Thienpont LM. Specifications for trueness and precision of a reference measurement system for serum/plasma 25-hydroxyvitamin D analysis. Clin Chim Acta 2009;408:8-13.

2. Clinical Laboratory Standards Institute. Preparation and validation of commutable frozen human serum pools as secondary reference materials for cholesterol measurement procedures (CLSI document C37-A). Wayne, PA: Clinical Laboratory Standards Institute. 1999.

3. Miller WG. Specimen materials, target values and commutability for external quality assessment (proficiency testing) schemes. Clin Chim Acta 2003;327:25-37.

4. European Committee of Standardization, International Organization for Standardization. In vitro diagnostic medical devices - Measurement of quantities in samples of biological origin - Metrological traceability of values assigned to calibrators and control materials (ISO/DIS 17511). Brussels. 2000.

5. Clinical Laboratory Standards Institute. Method comparison and bias estimation using patient samples (CLSI document EP-9A2). Wayne, PA: Clinical Laboratory Standards Institute. 2002.