CDC LABORATORY/MANUFACTURER HORMONE STANDARDIZATION (HOST) PROGRAM

STANDARDIZATION OF SERUM TOTAL TESTOSTERONE MEASUREMENTS

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GOALS

The objective of the Centers for Disease Control and Prevention's Hormone Standardization Project (CDC-HoSt Program) is to improve diagnosis, treatment, and prevention of diseases and disorders through the standardization of testosterone measurements.

PRINCIPLE

Standardization of total testosterone (TT) measurements in serum will be established through method comparison and bias estimation between the CDC Reference Laboratory and the testing laboratory. Single-unit, fresh-frozen serum samples will be used, and the observed bias will be compared to predefined limits. A laboratory is considered standardized to CDC 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 "Preparation and Validation of Commutable Frozen Human Serum"[1]. Sera prepared according to this protocol have been shown commutable in previous studies and were recommended for use in trueness control and calibration studies[2]. The materials underwent 2 freeze-thaw cycles and are within the range of total testosterone commonly observed in males and females in most adult populations.

All shipments will be made the first full weeks in **February**, **May**, **August**, and **November** for both Phase 1 and Phase 2 materials. Each laboratory must provide adequate frozen storage at or below -70°C. The participant must immediately transfer all CDC-HoSt program materials to a freezer for storage at -70°C upon receipt until use.

Procedure

The study consists of two phases:

In **Phase 1**, 40 samples, with 1.0 mL each, will be sent to the participant, with total testosterone concentrations assigned. The participating laboratory can use these 40 samples to perform a bias assessment and adjust its calibration as needed prior to the start of Phase 2. The CDC-HoSt Program will provide assistance on technical aspects of the measurement process, if requested, to help with adjusting the calibration. 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**, the laboratory will receive 4 sets (Phase 2A, 2B, 2C, and 2D) of 10 samples with unknown concentrations over the course of 12 months (quarterly shipments are made the first full weeks in February, May, August, and November). The samples in each set are to be analyzed on 2 different days in duplicate (n=4). A sample set consists of 2 vials, with 0.5 mL each vial, per sample (20 vials per set), using 1 sample vial per day. A total of 40 measurements will be made over 2 days and reported to the CDC. This will be repeated with each quarterly sample set. The laboratory's routine quality control procedures need to be followed during these analyses. Rejected runs need to be repeated. Prior to start of Phase 2 participants reportable range will be provided to the CDC. Only samples within the participant's reportable range will be sent.

Data Submission

Measurement results for each quarterly challenge are to be submitted to CDC within **four weeks** of the receipt of the samples to allow for data analysis and feedback prior to the next quarterly shipment. Data will be submitted to CDC through the provided data submission template to HoSt@cdc.gov. Individual measurements are to be reported in 3 significant figures and in ng/dL. Additional information about calibrators, reagents, and the instrument used will be required fields of the data submission template. The next shipment of materials will not be made without receipt of the previous data set and approval for shipment by the participant.

Reference Values

Reference values are assigned to the serum materials by the CDC reference method, which uses ID-HPLC/MS/MS and certified primary standards from the Australian National Measurement Institute (A-NMI).[3] The CDC reference method has been verified through comparison studies with the National Institute for Standards and Technology (NIST) and Dr. Linda Theinpont at the University of Ghent with methods that are recognized by the Joint Committee for Traceability in Laboratory Medicine (JCTLM) as a reference measurement procedures (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 to the participating laboratory in writing by CDC-HoSt Program 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, imprecision, and total error of the measurements will be assessed and compared to biological variability data. Results and conclusions from method comparison and bias estimation will be communicated to the participating laboratory in writing by CDC.

For certification purposes only an overall mean bias assessment will be used on the reported 40 samples. The 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] Values found below a participant's reportable range will not be used in the data assessment. Due to insufficient data for analysis a data set cannot be processed for certification if 2 or more samples out of the 40 are not reported, reported outside the reportable range, or removed as an outlier according to EP 9-A2. Feedback on performance will still be provided but certification cannot be issued.

The acceptable overall mean bias criterion of $\pm$6.4%, based on biological variability data, will be used to issue certifcation[6]. Certification is assumed for one year, and it needs to be renewed on an annual basis, with participation in Phase 2 only in subsequent years. CDC-HoSt Program can provide technical assistance to resolve problems in meeting the performance standards, thus ensuring the participant's long-term success in maintaining standardized testosterone measurements.

CDC-HoSt Program will issue to all participating laboratories within the established bias criterion annual certificates that document enrollment and performance in CDC-HoSt Program for total testosterone. Participation will remain anonymous, and with participants' approval, laboratories passing the predefined limits will be listed on the CDC Website (http://www.cdc.gov/labstandards/hs.html).

COLLABORATION FEES

Participation is voluntary. The fees associated with this process (samples, data processing, and reporting) will be covered by the participants Shipment costs will be covered by the participants by providing a FedEx account for shipment.

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)

Collaboration fees will be coordinated by and made to the CDC Foundation prior to the first shipment of samples each year.

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LOGISTICS

Upon CDC's 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, and contact person must be provided by the participant.

Phase 1 shipments will be made upon receipt of payment on the previously described shipment days.
Phase 2 shipments will be made 3 months after participants have obtained Phase 1 samples or when participants are ready. Shipments will be repeated quarterly for 12 months.

SAFETY

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

REFERENCES

1. 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.

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

3. Botelho JC, Shacklady C, Cooper HC, Tai SS-C, Van Uytfanghe K, Thienpont LM, Vesper HW. Isotope Dilution Liquid Chromatography/Tandem Mass Spectrometry Candidate Reference Method for Total Testosterone in Human Serum. Clin Chem 59:2 (2013).

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 EP9). Wayne, PA: Clinical Laboratory Standards Institute. 2002.

6. Yun YM, Botelho JC,¬¬ Chandler DW, Katayev A, Roberts WL, Stanczyk FZ, Vesper HW, Nakamoto JM, Garibaldi L, Clarke NJ, Fitzgerald RL. Performance Criteria for Testosterone Measurement Based on Biological Variation in Adult Males: Recommendations from PATH. Clinical Chemistry 58(12): 1703-1710, (2012).