Participant Protocol for CDC Hormone Standardization (CDC HoSt) Program Estradiol (E2)

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GOAL

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

PRINCIPLE

Standardization of estradiol (E2) measurements in serum is established through method comparison and bias estimation between the CDC reference measurement procedure (RMP) and the participating assay. Single-donor, frozen serum samples are used for compassion. The observed bias is compared to predefined criteria. An assay is considered standardized when the observed bias is within the predefined criteria.

PROTOCOL

Safety

Consider all serum specimens potentially positive for infectious agents including HIV and the hepatitis B virus. Hepatitis B vaccination series are recommended for all analysts working with whole blood and/or plasma. Universal precautions should be observed; wear protective gloves, laboratory coats, and safety glasses during all steps of this method. Any residual sample material should be discarded by autoclaving after analysis is completed. Place disposable plastic, glass, and paper (pipette tips, auto sampler vials, gloves, etc.) that contact serum in a biohazard autoclave bag and keep these bags in appropriate containers until sealed and autoclaved. Wipe down all work surfaces with appropriate disinfectant when work is finished.

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 estradiol commonly observed in males and females in most adult populations.

Logistics

In general, the materials are shipped during the first full week in **February**, **May**, **August**, and **November**. Shipping address, FedEx account number, and point of contact must be provided by the participant. Participants should assess the shipment for completeness, for samples that are damaged or leaking, and whether samples arrive frozen upon sample receipt. Participant must send an e-mail within 24 hours to confirm sample receipt and to report any discrepancies or problems (Standardization@cdc.gov).

A participant's reportable range should be provided to the CDC. Only samples within the participant's reportable range are provided, unless specific concentration range requests are made.

Each participant must utilize 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.

Process

The program consists of two phases:

In **Phase 1** (calibration phase), 40 samples (2 vials per sample, 0.5 mL per vial-80 vials total), are provided to the participant along with CDC reference values. The participants may use these 40 samples to perform a bias assessment and adjust calibration as needed prior to the start of Phase 2.

Phase 1 is optional for participants that have already completed internal comparisons to the reference laboratory and are satisfied with their performance. If needed, participants may request additional Phase 1 samples throughout enrollment. The CDC HoSt Program may assist on technical aspects of the measurement process, if requested.

In **Phase 2** (certification phase), 4 sets of samples are provided to the participant over the course of four quarters. Each sample set consists of 10 samples (2 vials per sample, 0.5 mL of per vial-20 vials total) and the participant analyzes each sample in duplicate measurement over two days (n=4). This is repeated with each quarterly sample set for a total of 40 samples at the end of the 4th quarter. The participating assay's routine quality control procedures need to be followed during analyses. Rejected runs need to be repeated.

The data is reported to the CDC quarterly. Evaluation reports are issued to the participant after each data submission. Data from 40 samples (four quarters) is needed for the initial certification evaluation. Participating assays are evaluated for certification quarterly after the initial certification evaluation. If certified, the certification is valid for one quarter and continuous enrollment is needed to keep continuous certification status. Participants may re-enroll annually. Certified assays are listed on the CDC website (https://www.cdc.gov/labstandards/csp/hs_host.html). Participants who wish not to be listed on the website may contact via email at standardization@cdc.gov.

Additionally, **120 Method Verification Samples** are available for more detailed method evaluation. These samples are typically used for method performance verification by assay manufacturers and developers. The 120 samples do not overlap with Phase 1 or Phase 2 samples. Altered samples may be included in the 120 Method Verification Sample kit.

Data Submission

Data for each **Phase 2** quarterly challenge should be submitted to the CDC within **four weeks** of the receipt of samples to allow for data analysis and feedback prior to the following quarterly challenge. Data must be submitted electronically to the CDC using provided data submission template. Individual measurements should be reported to 3 significant figures and in pg/mL. Additional information regarding calibrators, reagents, and the instrument used should be provided in the applicable fields of the data submission template.

Reference Values

Reference values are assigned to the serum materials by the CDC reference measurement procedure (RMP),[3] which uses ID-HPLC/MS/MS and certified primary standards from the National Metrology Institute of Japan (NMIJ). The CDC's RMP has been verified through comparison studies with the University of Ghent with a method that is recognized by the Joint Committee for Traceability in Laboratory Medicine (JCTLM) as an RMP of a higher order. Therefore, the materials are traceable as described in ISO 17511[4].

Data Analysis

Bias, imprecision, and total error of the measurements are assessed. Evaluation report including results and conclusions from method comparison are provided to the participant quarterly.

Method comparison and bias estimation is performed by the procedure described in the CLSI document EP09-A2 "Method Comparison and Bias Estimation Using Patient Samples."[5]

Following proposed criteria derived from biological variability data and data from epidemiologic studies in postmenopausal women are used for evaluation[6].

- allowable bias of $\pm$12.5 % for the samples with estradiol reference value >20 pg/mL
- allowable bias of $\pm$2.5 pg/mL for the samples with estradiol reference value ≤20 pg/mL

Mean bias of 40 samples is evaluated against the criteria. In addition, each sample is evaluated separately based on the criteria. Certification requires that mean bias of the 40 samples is within the criteria and 80% of the 40 reported samples, or 32 samples, meet the proposed bias criteria.

Certification is valid for one quarter and continuous participation is recommended. CDC HoSt Program provides technical assistance to resolve problems in meeting the performance standards as needed.

REFERENCES

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2. Miller WG. Specimen materials, target values and commutability for external quality assessment (proficiency testing) schemes. Clin Chim Acta 327 (2003) 25–37.
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6. Vesper HW, Botelho JC, Vidal ML, Rahmani Y, Thienpont LM, and Caudill SP. High variability in serum estradiol measurements in men and women. Steroids 82 (2014)7-13.