Longitudinal

Anti-Müllerian Hormone Collection

CODEBOOK

ARCHIVED DATASET 2019

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DESCRIPTION OF THE LONGITUDINAL AMH DATA SET

This codebook documents the release of the SWAN Anti-Müllerian Hormone (AMH) data from baseline through visit 10, and visits 12 and 13.

Who is included in the frozen dataset: 1536 women who were in the SWAN AMH ancillary study. To be eligible, women had to experience a natural menopause transition (i.e. without hysterectomy, bilateral oophorectomy or hormone therapy), have a documented date of their final menstrual period (FMP) and have at least one blood sample available for analysis while still pre- or early perimenopause. The dataset contains data from AMH and Inhibin B assays received from the Ansh lab.

Note: Ten samplings occurred before the actual baseline visit, resulting in a negative number for the sample day variable (SAMPDAY).

Brief description of the data: The goal of the AMH ancillary study was to measure AMH beginning while each subject was still pre- or early perimenopausal and then in all subsequent annual visits until (and including) the first visit occurring after the FMP. Because AMH is made by the granulosa cells in the ovary, which are depleted progressively during a woman's reproductive years, it was hypothesized that AMH levels would decline progressively and serve as a marker for predicting the FMP.

Description of Laboratory methods:

AMH was measured using the **picoAMH ELISA** from Ansh Labs (Webster, TX, USA).

Principle of the Test

The picoAMH ELISA is a quantitative three-step sandwich type immunoassay from Ansh Labs (Webster, TX) that is designed to measure human AMH. In the first step Calibrators, Controls and unknown samples are added to AMH antibody-coated microtiter wells and incubated. After the first incubation and washing, the wells are incubated with biotinylated AMH antibody solution. After the second incubation and washing, the wells are incubated with streptavidin horseradish peroxidase conjugate (SHRP) solution. After the third incubation and washing step, the wells are incubated with substrate solution (TMB) followed by an acidic stopping solution. In principle, the AMH antibody-biotin conjugate binds to the solid phase antibody-antigen complex which in turn binds to the streptavidin-enzyme conjugate. The antibody-antigen-biotin conjugate-SHRP complex bound to the well is detected by enzyme-substrate reaction. The degree of enzymatic turnover of the substrate is determined by dual wavelength absorbance measurement at 450 nm or 405 nm as primary test filter and 630 nm as reference filter. The absorbance measured is directly proportional to the concentration of AMH in the samples and calibrators. The picoAMH ELISA has an analytical measurable range of 2.0-11,000 pg/mL, with an analytical sensitivity of 1 pg/mL, and the assay employs recombinant human associated form of AMH as a calibrator. The method detects the full length and non-covalent complex forms of human AMH. There is no detectable cross-reactivity to other isoforms of AMH, different conformations of AMH, or other TGF-Beta superfamily hormones. The assay exhibits no interference by factors such as complement or heterophilic antibodies.

Calibration curve

PicoAMH ELISA calibrators were made in heat-inactivated serum matrix using recombinant pro-mature human AMH (BA 047, Ansh Labs, USA). The assay uses a seven point calibration (with blank subtracted). The log of AMH concentration is plotted on the X-axis, the log of matched optical density on the Y-axis, and the curve is fit using cubic regression (Gen V, Biotek

Instruments, USA, version 5.2). The kit calibration is traceable to manufacturer's working concentration.

Controls

Two kit controls based on recombinant human AMH antigen (BA 047, Ansh Labs, USA) spiked in serum (91.0 and 286.5 pg/mL) and two pooled human serum samples (8.5 and 20.4 pg/mL) were run in duplicate in every run. The nominal concentrations of the control samples were established by analyzing the samples in the picoAMH ELISA (AL-124, Ansh Labs, USA).

Imprecision

Reproducibility of the assay was determined on two serum controls and two kit controls with AMH concentration of 8.5 and 20.4, and 91.0 and 286.5 pg/mL, respectively. These controls were run in duplicates in 110 assay runs over 30 days. Precision was expressed as percent coefficient of variation (%CV) for total assay (n=110) variability. The within-assay and between-assay variation were calculated using CLSI EP5-A.

Assay Procedure

picoAMH ELISA is an enzymatically amplified two-site immunoassay. In the first step, 50 μL of assay buffer (protein-based TRIS buffer) was added to each of the microtitration wells coated with anti-AMH antibody. 100 μL of the reconstituted Calibrator and Controls (six vials, containing concentrations of approximately 0, 7.6, 31.0, 104.7, 360.2 and 1091.0 pg/mL of AMH in serum) and 1:2 diluted calibrator B (3.8 pg/mL) were added to the appropriate wells. 50 μL of samples were added using precision pipette to the sample designated wells followed by addition of 50 μL of Cal-124A/Sample-diluent to the sample added wells. The wells were incubated with shaking on an orbital microplate shaker for 3 hour at room temperature (~25°C). Then the plate was washed five times with an automated microplate washer using wash solution. In the second step, 100 μL of the AMH antibody-biotin conjugate in protein-based buffer) was added to each well and then incubated on an orbital shaker for one hour at room temperature. The wells were then washed five times as described above. In the third step, 100 μL of streptavidin labelled, horseradish peroxidase enzyme conjugates in protein-based buffer was added to each well. The wells were incubated on an orbital shaker for an additional 30 minutes at room temperature and washed five times as described above. After the final wash step, 100 μL of tetramethylbenzidine (TMB) substrate solution was added to each well and then incubated on an orbital shaker for 8-10 minutes. The color formation was stopped by addition of 100 μL stopping solution to each well. The degree of enzymatic turnover of the TMB was determined by dual wavelength absorbance measurement on a BioTek plate reader (BioTek instruments, Gen V, version 5.2, USA) at 450 or 405 nm as primary test filter and 630 nm as primary reference filter. The absorbance measured was directly proportional to the concentration of AMH in the samples. Calibrators were used to plot a calibration curve of absorbance versus AMH concentration. The AMH concentrations in the samples were then interpolated from the calibration curve.

Validation Procedures

The validation of the picoAMH assay was carried out following the manufacturer's (Ansh Labs Inc., Webster, TX, USA, picoAMH ELISA, cat # AL-124, Research use only) recommendations for preparation and storage of reagents, calibrators, and controls and for running the protocol.

Sensitivity

The analytical sensitivity in the assay as calculated by the interpolation of mean plus two standard deviation of 90 replicates of calibrator A (0 pg/mL) and 90 replicates of calibrator B (6.0 pg/mL) is 1.96 pg/mL.

Results Imprecision

Reproducibility of the picoAMH ELISA assay was determined using two human serum pools (QC1 and QC2) at 8.5 and 20.4 pg/mL and two kit controls (C1 and C2) at 91.0 and 286.5 pg/mL using one reagent lot. The total imprecision calculated on QC1, QC2 and C1, C2 over 110 assays (n=220) were 12.2 & 9.9 % and 4.6 & 4.9%, respectively.

Inhibin B was measured using the **Inhibin B ELISA** from Ansh Labs.

Summary and explanation

Inhibin B is a dimeric hormone that is composed of alpha (α) and beta B (βB) subunits. The free alpha subunits usually do not have any physiological effect. Therefore, the bioactivity of the inhibins depends on the formation of a dimeric α-β structure, and only dimeric forms of inhibins are biologically active. Inhibins are protein hormones secreted by granulosa cells of the ovary in the female and sertoli cells of the testis in the male. They selectively suppress the secretion of pituitary follicle stimulating hormone (FSH) and also have local paracrine actions in the gonads. Inhibin B levels have been reported in sertoli cell function (potential marker for spermatogenesis and testicular function), ovarian reserve and granulosa cell tumors.

Principle of the test

The Inhibin B ELISA is a quantitative three-step sandwich type immunoassay. In the first step Calibrators, Controls and unknown samples are added to Inhibin B antibody coated microtiter wells and incubated. After the first incubation and washing, the wells are incubated with biotinylated Inhibin B antibody. After the second incubation and washing, the wells are incubated with streptavidin horseradish peroxidase conjugate (SHRP). After the third incubation and washing step, the wells are incubated with substrate solution (TMB). After TMB incubation, an acidic stopping solution is added. In principle, the antibody-biotin conjugate binds to the solid phase antibody-antigen complex which in turn binds to the streptavidin-enzyme conjugate. The antibody-antigen-biotin conjugate-SHRP complex bound to the well is detected by enzyme-substrate reaction. The degree of enzymatic turnover of the substrate is determined by dual wavelength absorbance measurement at 450 nm as primary test filter and 630 nm as reference filter. The absorbance measured is directly proportional to the concentration of Inhibin B in the samples and calibrators.

Assay results data range

In the SWAN data set, AMH and Inhibin-B values that were noted as not detectable in the raw data were changed to a value of 1.45. Ansh provided the Coordinating Center with expected ranges of AMH by age groups. Expected ranges from Ansh are in the table below:

Serum Assay	Age group	Lower Limit (pg/mL)	Upper Limit (pg/mL)
picoAMH	25-40	190	9130
picoAMH	41-45	4.2	3300
picoAMH	46-50	4.03	539.9
picoAMH	51-55	6.7	29.4
picoAMH	53-70	Non Detectable	Non Detectable

picoAMH ELISA Bibliography:

picoAMH ELISA, AL-124

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8. **Su HI, Sammel MD, Homer MV, Bui K, Haunschild C, Stanczyk FZ.** Comparability of antimüllerian hormone levels among commercially available immunoassays. Fertility and Sterility. 2014 Jun; 101(6):1766-72.e1. [AMH ELISA AL-105, picoAMH ELISA AL-124]
9. **Welsh P, Smith K, Nelson SM.** A single-centre evaluation of two new Anti-Mullerian Hormone assays and comparison with the current clinical standard assay. Human Reproduction. 2014 May; 29(5):1035-41. [AMH ELISA AL-105, picoAMH ELISA-124]

Created Variables

- **SAMPDAY**: The number of days between the date the sample was obtained from the woman and her interview date at baseline. A positive value indicates that the sample was taken after the interview date.

List of Variables In The Data Set

Variable	Description	Code
ARCHID	Study ID Number	
VISIT	SWAN VISIT	
RACE	Race/Ethnicity	1= Black 2= Chinese/Chinese American 3= Japanese/Japanese American 4= White Non-Hispanic 5= Hispanic
SITE	SWAN STUDY SITE	11= Detroit, MI 12= Boston, MA 13= Chicago, IL 14= Oakland, CA 15= Los Angeles, CA 16= Newark, NJ 17= Pittsburgh, PA
SAMPDAY	Sample Day	# of days since baseline (day 0)
Ansh_Inhibin_B	Inhibin B	
Ansh_picoAMH	AMH	