DHEAS Assay

The DHEAS assay is a competitive immunoassay that was run on the Bayer Diagnostic ACS-180 automated analyzer using chemiluminescence technology. This assay was developed de novo for SWAN and uses rabbit polyclonal anti-DHEAS antibody, goat anti-rabbit IgG labeled with superparamagnetic particles (PMP), and DHEAS labeled with dimethylacridinuim ester (DMAE). Ten µL of serum is required for the assay in addition to sufficient dead volume for aspiration and repeat. The SWAN reporting range for the DHEAS assay is 0.04-11.5 µM (1.52-450 µg/dL). The actual assay range is 0.04-26.1 µM (1.52 to 1020 µg/dL). The assay is standardized against DHEAS obtained from Steraloids (Newport, RI). We report the following inter-assay and intra-assay coefficients of variation, 11.3% and 8.0%, respectively.

Testosterone (T) Assay

The testosterone (T) assay is a competitive immunoassay modified to run on the Bayer Diagnostic ACS-180 automated analyzer using chemiluminescence technology. The assay uses T labeled with DMAE, a polyclonal rabbit anti-T antibody, and a monoclonal mouse anti-rabbit antibody coupled to PMP. Forty-five µL of serum is required for the assay in addition to sufficient dead volume for aspiration and repeat assay determinations. The SWAN reporting range for the T assay is 0.4-3.5 nM (10-100 ng/dL). The actual assay range is 0.07-16.6 nM (2-478 ng/dL). The ACS testosterone assay is standardized analytically and confirmed by GC-MS. We report the following: inter-assay and intra-assay coefficients of variation, 11.3% and 4.6%, respectively.

Androstenedione (Adione) and Androstenediol (Adiol) Assays

Androstenedione (Adione) and 5-androstene-3β,17β-diol (Adiol) were analyzed in serum by radioimmunoassay (RIA) methods. The analytes were extracted from serum using hexane:ethyl acetate (3:2) and, following evaporation of the organic solvents, the extracts were redissolved in isooctane and applied to a Celite partition column impregnated with ethylene glycol. Adione and Adiol were eluted with isooctane and 40% toluene in isooctane, respectively. After evaporation of the eluates, each residue was reconstituted in assay buffer and appropriate aliquots were taken for RIA. Each RIA utilizes a highly specific antiserum in conjunction with an iodinated (Adione) or tritiated (Adiol) ligand. Following an appropriate incubation period, antibody-bound and unbound hormone were separated by use of either second antibody (anti-Adione) or dextran-coated charcoal for Adiol. The antibody-bound hormone was then counted after centrifugation. Inter-assay and intra-assay coefficients of variation were 8% and 10%, at 460 and 1450 pg/ml, respectively, for Adione, and 13% and 11% at 460 and 1210 pg/ml, respectively, for Adiol.

Estradiol (E2) Assay

The off-line estradiol (E2) assay is a semi-automated, competitive immunoassay with manual steps and an off-line incubation. Standards, quality control preparations, and serum samples are pipetted into 12x75 polypropylene tubes by hand with a dilution of antibody reagent, and then incubated. First, DMAE-labeled E2 and rabbit anit-E2/estradiol antibody are added to the tubes and incubated. Then all tubes are placed on Bayer Diagnostic's ACS-180 automated analyzer with monoclonal mouse anti-rabbit

IgG coupled to PMP for analysis. One mL of serum is required for the assay in addition to sufficient dead volume for aspiration and repeats. The SWAN reporting range for the E2 assay is 3.7-734 pM (1-200 pg/mL). The ACS E2-6 Master Curve standards are manufactured, standardized analytically and confirmed by GC-MS. We report the following inter-assay and intra-assay coefficients of variation, 13.8% and 8.5%, respectively.