

HABC Sex Steroid Hormone methods

Sex hormone binding globulin (SHBG), total testosterone, and dehydroepiandrosterone sulfate (DHEAS) were determined using an automated continuous, chemiluminescent immunoassay system (IMMULITE, Diagnostics Products Corporation, Los Angeles). The IMMULITE SHBG assay has a sensitivity of 0.20 nmol/L with a calibration range of 2-180 nmol/L. The total testosterone assay has a sensitivity of 8.7 ng/dL with a calibration range of 20-1600 ng/dL. The DHEAS assay has a sensitivity of 1.99 µg/dL with a calibration range of 30-1000 µg/dL. Measurements of higher concentrations were obtained by dilution of the original sample, when there was sufficient remaining sample. The inter-assay coefficients of variation (CV) calculated using 5% blind duplicate samples for the SHBG, total testosterone, and DHEAS assays were 49%, 18.1%, and 27.7% respectively. Part of the reason for the high CVs, however, is that all of these results are highly skewed. After log transformation of the data, the CVs were 9.7%, 8.2%, and 10.8%, respectively.

Serum concentrations of Free testosterone were measured using an enzyme immunoassay (EIA) kit (sensitivity = 0.19 pg/mL, detection range=0.25-100 pg/mL) from Diagnostic Systems Laboratories, Inc. (DSL, Webster, TX). This kit utilizes a rabbit anti-testosterone antiserum, which has low affinity for SHBG and albumin. All samples were measured in duplicate and the average of the two values was used for data analyses. The inter-assay coefficient of variation (CV) calculated using 5% blind duplicate samples, was 14%, and after log transformation of the data, 8.1%.