

# Third National Health and Nutrition Examination Survey 1988–1994

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## Documentation, Codebook, and Frequencies

**Surplus Sera Laboratory Component:**  
Racial/Ethnic Variation In Sex Steroid Hormone  
Concentrations Across Age In US Men

**Survey Years:**  
**1988 to 1991**

**SAS Export File:**  
**SSHORMON.XPT**



First Published: October 2006  
Last revised: N/A

# NHANES III Data Documentation

## Laboratory Assessment: Racial/Ethnic Variation in Sex Steroid Hormone Concentrations Across Age In US Men (NHANES III Surplus Sera)

Years of Coverage: 1988-1991

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### Introduction

It has been proposed that racial/ethnic variation in prostate cancer incidence may be, in part, due to racial/ethnic variation in sex steroid hormone levels. However, it remains unclear whether in the US population circulating concentrations of sex steroid hormones vary by race/ethnicity. To address this, concentrations of testosterone, sex hormone binding globulin, androstanediol glucuronide (a metabolite of dihydrotestosterone) and estradiol were measured in stored serum specimens from men examined in the morning sample of the first phase of NHANES III (1988-1991). This data file contains results of the testing of 1637 males age 12 or more years who participated in the morning examination of phase 1 of NHANES III and for whom serum was still available in the repository. Data Documentation for each of these four components is given in sections below.

## I. Testosterone

### Component Description

#### Summary

The androgen testosterone ( $17\beta$ -hydroxyandrost-4-en-3-one) has a molecular weight of 288 daltons. In men, testosterone is synthesized almost exclusively by the Leydig cells of the testes. The secretion of testosterone is regulated by luteinizing hormone (LH), and is subject to negative feedback via the pituitary and hypothalamus. Testosterone promotes the development of the secondary sex characteristics in men and serves to maintain the function of the prostate and seminal vesicles. Most of the circulating testosterone is bound to carrier proteins (SHBG = sex hormone-binding globulin). In women, small quantities of testosterone are formed in the ovaries. In physiological concentrations, androgens have no specific effects in women. Increased production of testosterone in women can cause virilization (depending on the increase). The determination of testosterone in women is helpful in the diagnosis of androgenic syndrome (AGS), polycystic ovaries (Stein-Leventhal syndrome) and when an ovarian tumor, adrenal tumor, adrenal hyperplasia or ovarian insufficiency is suspected. Testosterone

is determined in men when reduced testosterone production is suspected, e.g. in hypogonadism, estrogen therapy, chromosome aberrations (as in the Klinefelter's syndrome) and liver cirrhosis.

Elecsys Testosterone is based on a competitive test principle using a monoclonal antibody specifically directed against testosterone. Endogenous testosterone released from the sample by ANS (8-anilino-1-naphthalene sulfonic acid) and norgestrel competes with the added testosterone derivative labeled with ruthenium complex for the binding sites on the biotinylated antibody.

## Eligible Sample

Immunoassay for the in vitro quantitative determination of testosterone in human serum and plasma.

**Specimen collection and preparation** Only the specimens listed below were tested and found acceptable. Serum collected using standard sampling tubes or tubes containing separating gel. Li-, Na-, NH<sub>4</sub><sup>+</sup>-heparin, K3-EDTA, and sodium fluoride/potassium oxalate plasma. When sodium citrate is used, the results must be corrected by + 10%.

Criterion: Recovery within 90-110% of serum value or slope 0.9-1.1 + intercept within  $\leq \pm 2x$  analytical sensitivity (LDL) + coefficient of correlation  $> 0.95$ .

Stable for 1 week at 2-8°C, 6 months at -20°C. Freeze only once.<sup>5,6</sup>

Stability of serum obtained with tubes containing separating gel: 48 hours at 2-8°C (note the data provided by the tube manufacturer).

When processing samples in primary tubes, follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples. Do not use samples and controls stabilized with azide.

Ensure the patients' samples, calibrators, and controls are at ambient temperature (20-25°C) before measurement. Because of possible evaporation effects, samples, calibrators, and controls on the analyzers should be measured within 2 hours.

## Description of Laboratory

### Test principle

Competition principle. Total duration of assay: 18 minutes.

## Methodology

- 1st incubation: 50 µL of sample is incubated with a testosterone-specific biotinylated antibody and a testosterone derivative labeled with a ruthenium complex. The binding sites of the labeled antibody become occupied partly by the sample analyte (depending on its concentration) and partly by the ruthenium-labeled hapten to form the respective immunocomplexes.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell.

Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode

### Assay

Resuspension of the microparticles takes place automatically before use.

Read in the test-specific parameters via the reagent barcode. In exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

*Elecsys 2010:* Bring the cooled reagents to approx. 20°C and place on the reagent disk (20°C) of the analyzer. Avoid the formation of foam. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

### Calibration

**Traceability:** This method has been standardized via ID-GC/MS ("Isotope Dilution Gas Chromatography Mass Spectrometry").<sup>6,7</sup> Every testosterone reagent set has a barcoded label containing the specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer by the use of Elecsys Testosterone CalSet II.

**Calibration frequency:** Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:

*Elecsys 2010:*

- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)

**Laboratory  
Quality  
Control and  
Monitoring**

**Quality control**

For quality control, use Elecsys PreciControl Universal 1 and 2. Controls are run at begin of day, after every 100 samples and at the end of the day, at least once per reagent kit, and after every calibration. The ranges are based on the manufacturer's given range.

Beginning of day controls must fall within 2 SD to begin the run. We accept the controls if they fall within 3 SD only if the previous time was within 2 SD. This criteria applies to both controls within and across runs.

**Data  
Processing  
and Editing**

**Calculation**

The analyzer automatically calculates the analyte concentration of each sample (either in nmol/L, ng/mL or ng/dL).

Conversion factors: nmol/L x 0.288 = ng/mL

ng/mL x 3.47 = nmol/L

ng/mL x 100 = ng/dL

**Analytic  
Notes**

**Limitations - interference**

The assay is unaffected by icterus (bilirubin < 513 µmol/L or < 30 mg/dL), hemolysis (Hb < 1.1 mmol/L or < 1.8 g/dL), lipemia (triglycerides < 22.8 mmol/L or < 2000 mg/dL), and biotin < 123 nmol/L or < 30 ng/mL.

Criterion: Recovery within ± 10% of initial value.

In patients receiving therapy with high biotin doses (i.e. > 5 mg/day), no sample should be taken until at least 8 hours after the last biotin administration.

In vitro tests were performed on 17 commonly used pharmaceuticals.

No interference with the assay was found.

In isolated cases, elevated testosterone levels were seen in samples from female dialysis patients > 70 years old.

The risk of interference from potential immunological interactions between test components and rare sera has been minimized by the inclusion of suitable additives.

In rare cases interference due to extremely high titers of antibodies to ruthenium can occur.

Elecsys Testosterone contains additives which minimize these effects.

Extremely high titers of antibodies to streptavidin can occur in isolated cases and cause interference.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination

and other findings.

**Measuring range**

0.069-52.00 nmol/L or 0.020-15.00 ng/mL (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as < 0.069 nmol/L or < 0.020 ng/mL. Values above the measuring range are reported as > 52.00 nmol/L or > 15.00 ng/mL.

**Analytical sensitivity (lower detection limit)**

0.069 nmol/L (0.02 ng/mL)

The detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, within-run precision, n = 21).

**Functional sensitivity**

0.42 nmol/L (0.12 ng/mL)

The functional sensitivity is the lowest analyte concentration that can be reproducibly measured with a between-run coefficient of variation of less than or equal to 20%.

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## II. Sex hormone-binding globulin (SHBG)

### Component Description

Sex hormone-binding globulin (SHBG) is the blood transport protein for testosterone and estradiol. It is a large glycoprotein with a molecular weight of about 95 kD, and exists as a homodimer composed of two identical subunits. Each subunit contains two disulfide bridges.<sup>1</sup> Planar C18 and C19 steroids with a 17 $\alpha$ -hydroxyl group bind particularly well,<sup>2,3</sup> whereas C19 17-ketosteroids such as dehydroepiandrosterone (DHEA) and androstendione do not bind so easily. SHBG has a high binding affinity to dihydrotestosterone (DHT), medium affinity to testosterone and estradiol, and only a low affinity to estrone, DHEA, androstendione, and estriol. SHBG binds reversibly to sexual steroids. Albumin, which exists in far higher concentrations than SHBG, also binds sexual steroids – although with a clearly lower binding affinity (e.g. about 100 times lower for testosterone). SHBG has a half-life of about 7 days and is produced mainly by the liver. Its synthesis and secretion are regulated by estrogen.<sup>4,5</sup> SHBG serum concentrations depend on the extent, duration, and the kind of estrogen applied, and how regulation takes place. Androgens and gestagens with androgenic residual action have the opposite effect. In the serum SHBG mainly takes over the transportation of steroids and the reduction/regulation of the effect of androgen.<sup>6,7</sup> Decreased SHBG serum levels are associated with conditions where elevated androgen levels are present or where the effect of androgen on its target organs is excessive. This explains the gender-related differences seen between men and women, especially during puberty.

Measurement of SHBG can be an important indicator of an excessive/chronic androgenic action where androgen levels are normal, but where clinical symptoms would seem to indicate androgen in excess. SHBG is a useful supplementary parameter in the determination of androgen where a relatively high concentration of free androgen (e.g. testosterone) is suspected.<sup>8</sup>

By calculating the free androgen index (FAI), also called free testosterone index (FTI), from the ratio of total testosterone (TT) to SHBG [% FAI or  $FTI = (TT/SHBG) * 100$ ], it is possible to calculate the approximate amount of free testosterone (FTc), as there is a direct correlation between FAI and FT. By additionally taking the non-specifically albumin-bound testosterone into account, it is possible to calculate the bioavailable testosterone (BATc), which is the sum of free testosterone and the albumin-bound testosterone fraction, calculated via the association constant to albumin. Only free testosterone is biologically active, and it best indicates the clinical situation of the patient. Free and bioavailable testosterone are also referred to as non-SHBG-bound testosterone and can be obtained by precipitation of the SHBG-bound-testosterone with ammonium sulfate, and by equilibrium

dialysis.<sup>9,10</sup>

Elevated SHBG levels can be seen in elderly men, and are often found in patients with hyperthyroidism and cirrhosis of the liver. SHBG levels also increase when oral contraceptives or antiepileptic drugs are taken. Pregnant women have markedly higher SHBG serum concentrations due to their increased estrogen production.

Decreased SHBG concentrations are often seen with hypothyroidism, polycystic ovarian syndrome (PCOS), obesity, hirsutism, elevated androgen levels, alopecia, and acromegaly.

Elecsys SHBG employs two monoclonal antibodies specifically directed against human SHBG. Cross-reactivity with  $\alpha$ 1-fetoprotein (AFP), corticosteroid binding globulin (CBG), DHT, estradiol, fibrinogen, human immunoglobulin A (IgA), human immunoglobulin G (IgG), plasminogen, thyroxine binding globulin (TBG), testosterone, thyroglobulin (Tg), transferrin, and thyrotropin (TSH) is negligible.

## Eligible Sample

Only the specimens listed below were tested and found acceptable. Serum collected using standard sampling tubes or tubes containing separating gel, or lithium heparin plasma.

Criterion: Recovery within 90-110% of serum value or slope 0.9-1.1 + intercept within  $\leq \pm 2x$  analytical sensitivity (LDL) + coefficient of correlation  $> 0.95$ .

Stable for 3 days at 2-8°C, 1 month at -20°C. Freeze only once.<sup>11</sup>

When processing samples in primary tubes, follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples. Do not use samples and controls stabilized with azide.

Ensure the patients' samples, calibrators, and controls are at ambient temperature (20-25°C) before measurement. Because of possible evaporation effects, samples, calibrators, and controls on the analyzers should be measured within 2 hours.

## Description of Laboratory Methodology

### Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 10  $\mu$ L of sample, a biotinylated monoclonal SHBG-

specific antibody, and a monoclonal SHBG-specific antibody labeled with a ruthenium complex form a sandwich complex.

- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.

- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell.

Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

### **Assay**

Resuspension of the microparticles takes place automatically before use.

Read in the test-specific parameters via the reagent barcode. In exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

*Elecsys 2010:* Bring the cooled reagents to approx. 20°C and place on the reagent disk (20°C) of the analyzer. Avoid the formation of foam. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

### **Calibration**

**Traceability:** This method has been standardized against the 1st International Standard for SHBG from the National Institute for Biological Standards and Control (NIBSC) code 95/560.11

Every SHBG reagent set has a barcoded label containing the specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer by the use of Elecsys SHBG CalSet.

*Calibration frequency:* Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:

*Elecsys 2010:*

- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)

## **Laboratory Quality Control and**

### **Quality control**

For quality control, use Elecsys PreciControl Universal 1 and 2.

Controls are run at begin of day, after every 100 samples and at the

## Monitoring

end of the day, at least once per reagent kit, and after every calibration. The ranges are based on the manufacturer's given range.

Beginning of day controls must fall within 2 SD to begin the run. We accept the controls if they fall within 3 SD only if the previous time was within 2 SD. This criteria applies to both controls within and across runs.

## Data Processing and Editing

### Calculation

The analyzer automatically calculates the analyte concentration of each sample either in nmol/L, µg/mL or mg/L (selectable).

Conversion factors: nmol/L x 0.095 = µg/mL (mg/L)

µg/mL (mg/L) x 10.53 = nmol/L.

## Analytic Notes

### Limitations - interference

The assay is unaffected by icterus (bilirubin < 1026 µmol/L or < 60 mg/dL), hemolysis (Hb < 1.8 mmol/L or < 2.9 g/dL), lipemia (Intralipid < 2700 mg/dL), and biotin < 60 ng/mL.

Criterion: Recovery within ± 10% of initial value.

In patients receiving therapy with high biotin doses (i.e. > 5 mg/day), no sample should be taken until at least 8 hours after the last biotin administration. No interference was observed from rheumatoid factors up to a concentration of 1160 IU/mL.

There is no high-dose hook effect at SHBG concentrations up to 1000 nmol/L.

In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found.

As with all tests containing monoclonal mouse antibodies, erroneous findings may be obtained from samples taken from patients who have been treated with monoclonal mouse antibodies or have received them for diagnostic purposes.

In rare cases interference due to extremely high titers of antibodies to ruthenium can occur.

Elecsys SHBG contains additives which minimize these effects.

Extremely high titers of antibodies to streptavidin can occur in isolated cases and cause interference.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

### Measuring range

0.350-200 nmol/L (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as < 0.350 nmol/L.

Values above the measuring range are reported as > 200 nmol/L.

**Analytical sensitivity (lower detection limit)**

0.35 nmol/L

The detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, within-run precision, n = 21).

**Method comparison**

A comparison of Elecsys SHBG (y) with a commercially available SHBG test (x) using clinical samples gave the following correlations:

Number of samples measured: 109

Passing/Bablok12 Linear regression

$$y = 1.17x - 3.26 \quad y = 1.15x - 1.82$$

$$\square = 0.909 \quad r = 0.981$$

$$SD (md68) = 3.24 \quad Sy.x = 4.15$$

The sample concentrations were between approx. 11.2 and 155 nmol/L.

**Analytical specificity**

For the monoclonal antibodies used, non detectable cross-reactivities were found for the following substances:

AFP, CBG, DHT, estradiol, fibrinogen, human IgA, human IgG, plasminogen, TBG, testosterone, Tg, transferrin, and TSH.

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### III. Androstanediol glucuronide

#### Component Description

3 $\alpha$ -androstanediol glucuronide (3 $\alpha$ -diol G, 5 $\alpha$ -Androstane-3 $\alpha$ ,17 $\beta$  diol glucuronide) is the glucuronide conjugate of 3 $\alpha$ -androstanediol, a major metabolite of dihydrotestosterone (DHT) <sup>[1]</sup>. 3 $\alpha$ -diol and 3 $\alpha$ -diol G are C19 steroids; the presence of a 17-hydroxyl group (retained from testosterone and DHT) determines their androgenic potency. In bioassays, the potency of 3 $\alpha$ -diol is estimated to be as much as 75% that of testosterone <sup>[2]</sup>. 3 $\alpha$ -diol is the product of intracellular reduction of DHT, which is enzymatically mediated by 3 $\beta$ -hydroxysteroid dehydrogenase <sup>[1,3-7]</sup>. A significant proportion of serum 3 $\alpha$ -diol may also be derived from dehydroepiandrosterone sulfate and  $\Delta$ 4-androstenedione. Although the sites of production are not completely defined <sup>[3,4,5]</sup>, it appears that most of the circulating 3 $\alpha$ -diol in women is derived directly or indirectly from adrenal androgen secretion <sup>[1,8]</sup>. Similarly, the site(s) of 3 $\alpha$ -diol glucuronidation has not been conclusively defined; postulated sites include the skin and liver <sup>[1,6,7]</sup>. 3 $\alpha$ -diol G may also be synthesized directly from glucuronidated precursors, including androsterone glucuronide <sup>[1]</sup>. Glucuronide conjugation of 3 $\alpha$ -diol appears to facilitate inactivation and urinary excretion of this compound. The measurement of 3 $\alpha$ -diol G has been advocated as a means of assessing skin androgen activity <sup>[1,6,7]</sup>. The androgen sensitivity of skin is dependent upon the presence of the 5 $\alpha$ -reductase enzyme, which converts testosterone to DHT. Evidence suggests that it is DHT binding to the androgen receptor which mediates skin androgen bioactivity. Although 3 $\alpha$ -diol and 3 $\alpha$ -diol G do not appear to have significant direct androgenic action, these compounds may reflect testosterone and DHT production <sup>[9]</sup>. Furthermore, interconversion of 3 $\alpha$ -diol and DHT can occur <sup>[1,6,7]</sup>, and this may account for the

relative biopotency of 3 $\alpha$ -diol G mentioned above. Elevated levels of serum 3 $\alpha$ -diol G have been reported in congenital adrenal hyperplasia [10,11] and in various forms of female hirsutism [1,6,7], including idiopathic hirsutism, and polycystic ovary disease. In some cases, serum levels of 3 $\alpha$ -diol G are elevated even in the presence of normal levels of other serum androgens [1,6,7]. A decline in serum 3 $\alpha$ -diol G levels has been reported with dexamethasone administration in hirsute women [8], and measurements of 3 $\alpha$ -diol G may be useful in tests of androgen metabolism [12]. Therefore, measurement of serum 3 $\alpha$ -diol G levels may be useful in the clinical evaluation and management of hirsutism and other conditions with excessive adrenal androgen production. Previous assays for 3 $\alpha$ -diol G require prior sample extraction and preparation; furthermore, 3 $\alpha$ -diol G levels reported in early literature may have been overestimated due to technical inadequacies [1]. Unlike other 3 $\alpha$ -diol G immunoassays, the DSL method does not require prior sample extraction [1,12-14].

**Eligible Sample**

The DSL-10-9200 ACTIVE® Androstenediol Glucuronide Enzyme Immunoassay (EIA) Kit provides materials for the quantitative measurement of androstenediol glucuronide in serum. This assay is intended for *in vitro* diagnostic use. Serum should be used and the usual precautions for venipuncture should be observed. Specimens may be stored at 2-8°C for up to 24 hours and should be frozen at -20°C or lower for longer periods. Do not use hemolyzed or lipemic specimens.

**Description of Laboratory Methodology**

The procedure follows the basic principle of enzyme immunoassay where there is competition between an unlabeled antigen and an enzyme-labeled antigen for a fixed number of antibody binding sites. The amount of enzyme-labeled antigen bound to the antibody is inversely proportional to the concentration of the unlabeled analyte present. Unbound materials are removed by decanting and washing the wells.

**REAGENTS SUPPLIED**

**A. GARG-Coated Microtitration Strips:** One strip holder containing 96 microtitration wells coated with goat anti-rabbit gamma globulin serum. Store at 2-8°C until expiration date in the resealable pouch with a desiccant to protect from moisture.

- B. B. 3 $\alpha$ -Diol G Standards:** One vial, 2.0 mL, labeled A containing 0 ng/mL and five vials, 1.0 mL each, labeled B - F, containing concentrations of approximately 0.5, 2.5, 10.0, 30.0 and 100.0 ng/mL androstenediol glucuronide in a protein-based (BSA) buffer with a non-mercury preservative. Refer to vial labels for exact concentrations. Store opened vials at 2-8°C for up to two weeks. For longer periods, store at -20°C or lower until expiration date. *STANDARDIZATION NOTE: Due to the lack of universally accepted material, the reference preparation of the 3 $\alpha$ -Diol Glucuronide Standards & Controls was obtained from GroPep, Australia, purified by HPLC, purity verified (single spot) by Thin Layer Chromatography and performance verified by immunoassay.*
- C. 3 $\alpha$ -Diol G Controls:** Two vials, 1.0 mL each, Levels I and II, containing low and high concentrations of androstenediol glucuronide in protein-based (BSA) buffer with a non-mercury preservative. Refer to vial labels for exact concentrations. Store opened vials at 2-8°C for up to two weeks. For longer periods, store at -20°C or lower until expiration date.
- D. 3 $\alpha$ -Diol G Enzyme Conjugate Concentrate:** One vial, containing 0.3 mL of a solution of androstenediol glucuronide conjugated to horseradish peroxidase in a protein-based (BSA) buffer with a non-mercury preservative. Dilute prior to use in Conjugate Diluent. Store at 2-8°C until expiration date. *NOTE: The dilution of this reagent should be made prior to use in the assay.*
- E. 3 $\alpha$ -Diol G Conjugate Diluent:** One vial, 6 mL, containing a protein-based (BSA) buffer with a non-mercury preservative. Store at 2-8°C until expiration date.
- F. 3 $\alpha$ -Diol G Antiserum:** One vial, 11 mL, containing anti-androstenediol glucuronide antiserum in a protein-based (BSA) buffer with a non-mercury preservative. Store at 2-8°C until expiration date.
- G. TMB Chromogen Solution:** One vial, 11 mL, containing a solution of tetramethylbenzidine (TMB) in citrate buffer with hydrogen peroxide. Store at 2-8°C until expiration date.
- H. Wash Concentrate:** One bottle, 60 mL, containing buffered saline with a nonionic detergent. Store at 2-8°C or room temperature until expiration date. Dilute 25-fold with deionized water prior to use.
- I. Stopping Solution:** One vial, 11 mL, containing 0.2 M sulfuric acid. Store at 2-8°C or room temperature until expiration date. *NOTE: All reagents and samples must be allowed to reach room temperature (~25° C) and mixed thoroughly by gentle inversion before use.*

## Laboratory Quality Control and Monitoring

Two levels of control are provided with the kit. We run each control on a plate. Controls and samples are done in duplicate. The manufacturer's range is used as a guideline until we have at least 20 data points to set our own mean and SD.

If both controls are within 2 SD – we accept the data. If one control is within 2 SD and the other within 3 SD – we accept the data. If one or both controls are outside 3 SD – we do not accept the results and repeat the plate.

## Data Processing and Editing

### RESULTS

A. Calculate the mean absorbance for each Standard, Control or unknown.

B. Using a linear-log graph paper, plot the mean absorbance readings for each of the standards along the y-axis versus the androstenediol glucuronide concentrations in ng/mL along the x-axis. Alternatively, any data reduction software designed for immunoassays could be used. Four-parameter curve-fit is recommended.

C. Draw the best fitting curve through the mean of the duplicate points.

D. Determine the androstenediol glucuronide concentrations of the Controls and unknowns from the standard curve by matching their mean absorbance readings with the corresponding androstenediol glucuronide concentrations. *If the absorbance reading for the first standard exceed the limitations of the plate reader, a second reading at 405 nm is needed (reference filter 600 or 620 if available). In this case, proceed to construct a second standard curve as above with the absorbance readings of all Standards at 405 nm. The concentration of the off-scale samples at 450 nm are then read from the new standard curve. The readings at 405 nm should not replace the on-scale readings at 450.*

We use Softmax Pro as our data reduction software.

## Analytic Notes

### EXPECTED VALUES

Normal ranges should be established by each laboratory. Results of a study conducted by an independent laboratory with the DSL-6000 3 $\alpha$ -Diol G RIA are reported below: POPULATION N MEAN  $\pm$  1 SD (ng/mL)

ABSOLUTE RANGE (ng/mL) Premenopausal Women 0.5 - 5.4  
Postmenopausal Women 0.1 - 6.0 Hirsute Women 1.3 - 9.4 Men  
3.4 - 22.0

**PERFORMANCE CHARACTERISTICS** All performance characteristics are stated in ng/mL.

- A. Sensitivity:** The theoretical sensitivity, or minimum detection limit, as calculated by interpolation of the mean minus two standard deviations of 8 replicates of the 0 ng/mL 3 $\alpha$ -Diol G Standard, is 0.33 ng/mL.
- B. Precision:** The intra-assay precision was determined from the mean of 16 replicates each. 3.0 ng/mL - 6.7%, 9.6 ng/mL - 5.2%, 29.6 ng/mL - 6.1% The inter-assay precision was determined from the mean of duplicates for 3 samples in 10 separate runs. 2.5 ng/mL - 12.0%, 9.2 ng/mL - 4.3%, 28.7 ng/mL - 5.6%
- C. Recovery:** Three serum samples containing different levels of endogenous androstanediol glucuronide were spiked with different amounts of androstanediol glucuronide and assayed. Recoveries ranged from 87 to 110% with an average recovery of 96%.

**Linearity:** Three serum samples were diluted with 0 pg/mL androstanediol glucuronide Standard and assayed. Samples were diluted 1:2 1:4 1:8 and 1:16. Recoveries ranged from 85 to 108% with an average recovery of 101%.

**Specificity:** The cross-reactivity of the 3 $\alpha$ -diol G antiserum has been measured against various compounds. The percent cross-reactivity is expressed as the ratio of the 3 $\alpha$ -diol G concentration to the concentration of the reacting compound at 50% binding of the 0 ng/mL 3 $\alpha$ -Diol G Standard. RESULTS: 5 $\alpha$ -Androstan-3 $\alpha$ ,17 $\beta$ -diol,17-glucuronide - 100% 5 $\alpha$ -Dihydrotestosterone glucuronide - 1.2% Testosterone 17-glucuronide triacetylmethylester - 0.9% The rest of the compounds listed were non-detectable: 5 $\alpha$ -Androstane-3 $\alpha$ -ol-17-one// 5 $\alpha$ -Androstane-3 $\alpha$ ,17 $\beta$ -diol 3-glucuronide// Testosterone glucuronide// Testosterone// 11 $\beta$ -Hydroxytestosterone// 5 $\alpha$ -Dihydrotestosterone// 5 $\beta$ -Dihydrotestosterone// 5 $\alpha$ -Androstane-3 $\alpha$ ,17 $\beta$ -diol 3-glucuronide triacetylmethylester// 5 $\alpha$ -Androstan-3 $\alpha$ -ol-17-one glucuronide// 5 $\alpha$ -Androstane-3 $\beta$ ,17 $\beta$ -diol// 5 $\alpha$ -Androstane-3,17-dione// 5 $\beta$ -Androstane-3,17-dione// 4-Androstene-3,17-dione// 5 $\beta$ -Androstane-3 $\beta$ ,17 $\beta$ -diol// Dehydroepiandrosterone// Cortisol// Corticosterone// Progesterone// Estrone// Estradiol-17 $\beta$ .

**Method Comparison:** The DSL-10-9200 ACTIVE® Androstenediol Glucuronide EIA has been compared to a commercially available RIA kit [Method X]. One hundred and seven samples were assayed and linear regression analysis of the results yielded the following: n = 107 Means: DSL-10-9200 = 4.5 DSL-6000 = 4.2 Regression: [DSL-10-9200] = 0.99 [Method X] + 0.08 r = 0.94

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## IV. Estrogens

### Component Description

Estrogens are responsible for the development of the secondary female sex characteristics. Together with gestagens they control all the important female reproductive processes. The biologically most active estrogen is 17  $\beta$  -estradiol. This is a steroid hormone having a molecular weight of 272 daltons. Estrogens are produced primarily in the ovary (follicle, corpus

luteum), but small quantities are also formed in the testes and in the adrenal cortex. During pregnancy, estrogens are mainly formed in the placenta. About 98% of estradiol is bound to transport proteins (SHBG = sex hormone binding globulin). Estrogen secretion is biphasic during the menstrual cycle. The determination of estradiol is utilized clinically in the elucidation of fertility disorders in the hypothalamus-pituitary-gonad axis, gynecomastia, estrogen-producing ovarian and testicular tumors and in hyperplasia of the adrenal cortex. Further clinical indications are the monitoring of fertility therapy and determining the time of ovulation within the framework of in vitro fertilization (IVF).

Elecsys Estradiol II employs a competitive test principle using a polyclonal antibody specifically directed against 17  $\beta$  -estradiol. Endogenous estradiol released from the sample by mesterolone competes with the added estradiol derivative labeled with a ruthenium complex for the binding sites on the biotinylated antibody.

## **Eligible Sample**

Immunoassay for the in vitro quantitative determination of estradiol in human serum and plasma. The electrochemiluminescence immunoassay "ECLIA" is intended for use on the Roche Elecsys 1010/2010 and MODULAR ANALYTICS E170 (Elecsys module) immunoassay analyzers.

### **Specimen collection and preparation**

Only the specimens listed below were tested and found acceptable: Serum collected using standard sampling tubes or tubes containing separating gel. Li-, Na-, NH<sub>4</sub><sup>+</sup>-heparin, K<sub>3</sub>-EDTA, sodium citrate, and sodium fluoride/potassium oxalate plasma.

Criterion: Recovery within 90-110% of serum value or slope 0.9-1.1 + intercept within  $\pm 2x$  analytical sensitivity (LDL) + coefficient of correlation  $> 0.95$ .

Stable for 2 days at 2-8°C, 6 months at -20°C. Freeze only once.<sup>6,7</sup>

When processing samples in primary tubes, follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples. Do not use samples and controls stabilized with azide.

Ensure the patients' samples, calibrators, and controls are at ambient temperature (20-25°C) before measurement.

Because of possible evaporation effects, samples, calibrators, and controls on the analyzers should be measured within 2 hours.

## Description of Laboratory Methodology

### Test principle

Competition principle. Total duration of assay: 18 minutes.

- 1st incubation: By incubating the sample (35  $\mu$ L) with an estradiol-specific biotinylated antibody, an immunocomplex is formed, the amount of which is dependent upon the analyte concentration in the sample.
- 2nd incubation: After addition of streptavidin-coated microparticles and an estradiol derivative labeled with a ruthenium complex, the still-vacant sites of the biotinylated antibodies become occupied, with formation of an antibody-hapten complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell.

Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

### Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated. All information required for correct operation is read in via the respective reagent barcodes.

### Storage and stability

Store at 2-8°C.

Store the Elecsys Estradiol II reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

### Assay

Resuspension of the microparticles takes place automatically before use.

Read in the test-specific parameters via the reagent barcode. In exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

*Elecsys 2010:* Bring the cooled reagents to approx. 20°C and place on the reagent disk (20°C) of the analyzer. Avoid the formation of foam. The system **automatically** regulates the temperature of the reagents and the opening/closing of the bottles.

### Calibration

Traceability: This method has been standardized via ID-GC/MS (“isotope dilution-gas chromatography/mass spectrometry”).<sup>7,8</sup> Every Estradiol II reagent set has a barcoded label containing the specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer by the use of Elecsys Estradiol II CalSet II.

*Calibration frequency:* Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:

*Elecsys 2010:*

- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)

*For all analyzers:*

- as required: e.g. quality control findings outside the specified limits

*Calibration verification:* Not necessary. The analyzer’s software automatically checks the validity of the curve and draws attention to any deviations.

## Laboratory Quality Control and Monitoring

### Quality control

For quality control, use Elecsys PreciControl Universal 1 and 2. Controls are run at begin of day, after every 100 samples and at the end of the day, at least once per reagent kit, and after every calibration. The ranges are based on the manufacturer’s given range.

Begin of day controls must fall within 2 SD to begin the run. We accept the controls if they fall within 3 SD only if the previous time was within 2 SD. This criteria applies to both controls within and across runs.

## Data Processing and Editing

### Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in pmol/L, pg/mL, ng/L or additionally in nmol/L with E170).

Conversion factors: pmol/L x 0.273 = pg/mL (ng/L)

pg/mL x 3.67 = pmol/L

### Limitations - interference

The assay is unaffected by icterus (bilirubin < 1129 µmol/L or < 66 mg/dL), hemolysis (Hb < 0.621 mmol/L or < 1.0 g/dL), lipemia (Intralipid < 1000 mg/dL), and biotin < 147 nmol/L or < 36 ng/mL.

Criterion: Recovery within ± 10% of initial value.

In patients receiving therapy with high biotin doses (i.e. > 5 mg/day), no sample should be taken until at least 8 hours after the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 1200 IU/mL.  
In vitro tests were performed on 18 commonly used pharmaceuticals.  
No interference with the assay was found.  
The risk of interference from potential immunological interactions between test components and rare sera has been minimized by the inclusion of suitable additives.

## **Analytic Notes**

Erroneous test results may be obtained from samples taken from patients who have been exposed to vaccines containing rabbit serum or when keeping rabbits as pet animals.  
In rare cases, interference due to extremely high titers of antibodies to streptavidin and ruthenium can occur.  
Elecys Estradiol II contains additives which minimize these effects.  
For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

### **Measuring range**

18.4-15,781 pmol/L (5.00-4300 pg/mL) (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as < 18.4 pmol/L or < 5.00 pg/mL. Values above the measuring range are reported as > 15,781 pmol/L or > 4300 pg/mL (or up to 78,905 pmol/L or 21,500 pg/mL for 5-fold diluted samples).

### **Analytical sensitivity (lower detection limit)**

18.4 pmol/L (5.0 pg/mL)

The detection limit represents the lowest analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, within-run precision, n = 21).

### **Functional sensitivity**

44 pmol/L (12 pg/mL)

The functional sensitivity is the lowest analyte concentration that can be reproducibly measured with a between-run coefficient of variation of  $\leq 20\%$ .

## **References**

### **References**

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## NCHS Locator Fields

**Title:** Racial/Ethnic Variation in Sex Steroid Hormone Concentrations Across Age In US Men

**Contact Number:** 1-866-441-NCHS

**Years of Content:** 1988-1991

**First Published:** October 2006

**Revised:** N/A

**Access Constraints:** None

**Use Constraints:** None

**Geographic Coverage:** National

**Subject:** Male Sex Steroid Concentrations

**Record Source:** NHANES III (1988-1991), phase 1 morning sample

**Survey Methodology:** NHANES III is a stratified multistage probability sample of the civilian non-institutionalized population of the U.S.

**Medium:** NHANES Web site; SAS transport files

**National Health and Nutrition Examination Survey  
Codebook for Data Production (1988-1991)  
(NHANES III)**

**Racial/Ethnic Variation In Sex Steroid Hormone Concentrations  
Across Age In US Men (SSHORMON)  
Person Level Data**

First Published: October 2006

Last Revised: N/A



<b>SEQN</b>	<b>Target</b>
	M(12 Yrs. to 150 Yrs.)
<b>Hard Edits</b>	<b>SAS Label</b>
	Respondent sequence number
<b>English Text:</b> Respondent sequence number.	
<b>English Instructions:</b>	

<b>SSE2</b>	<b>Target</b>
	M(12 Yrs. to 150 Yrs.)
<b>Hard Edits</b>	<b>SAS Label</b>
	Estrodiol (pg/mL)
<b>English Text:</b> Estrodiol (pg/mL).	
<b>English Instructions:</b>	

Code or Value	Description	Count	Cumulative	Skip to Item
7.61 to 175.8	Range of Values	1628	1628	
.	Missing	8	1636	

<b>SSSHBG</b>	<b>Target</b>
	M(12 Yrs. to 150 Yrs.)
<b>Hard Edits</b>	<b>SAS Label</b>
	Sex hormone-binding globulin (nmol/L)
<b>English Text:</b> Sex hormone-binding globulin (nmol/L).	
<b>English Instructions:</b>	

Code or Value	Description	Count	Cumulative	Skip to Item
5 to 199.2	Range of Values	1621	1621	
.	Missing	15	1636	

<b>SSTESTO</b>	<b>Target</b>			
	M(12 Yrs. to 150 Yrs.)			
<b>Hard Edits</b>	<b>SAS Label</b>			
	Testosterone (ng/mL)			
<b>English Text:</b> Testosterone (ng/mL).				
<b>English Instructions:</b>				
<b>Code or Value</b>	<b>Description</b>	<b>Count</b>	<b>Cumulative</b>	<b>Skip to Item</b>
0.05 to 13.82	Range of Values	1625	1625	
.	Missing	11	1636	

<b>SSAAG</b>	<b>Target</b>			
	M(12 Yrs. to 150 Yrs.)			
<b>Hard Edits</b>	<b>SAS Label</b>			
	3 alpha androstanediol glucuronide ng/mL			
<b>English Text:</b> 3 alpha androstanediol glucuronide (ng/mL).				
<b>English Instructions:</b>				
<b>Code or Value</b>	<b>Description</b>	<b>Count</b>	<b>Cumulative</b>	<b>Skip to Item</b>
0.093 to 182.539	Range of Values	1617	1617	
.	Missing	19	1636	