



## Testosterone

### Salivary Testosterone Measurement in Wave I of the Social Life Health & Aging Project

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#### Rationale

Testosterone, a steroid hormone derived from cholesterol, occurs more abundantly in circulation among men, than women (Guyton 1991). Testosterone is synthesized in the testes among males, the ovaries among females, and possibly the adrenal glands in both sexes (Guyton 1991; Davis and Tran 2001), though the physiological mechanism of testosterone synthesis and excretion in women is not completely understood (Lobo 2001). During the aging process, testosterone levels diminish gradually in both sexes (Tenover 1997; Davis and Tran 2001; Lobo 2001; Ellison, Bribiescas et al. 2002; Feldman, Longcope et al. 2002) and without an abrupt decline during menopause (Davis and Tran 2001; Lobo 2001). Surgical menopause due to premenopausal oophorectomy results in abrupt decline in

testosterone (Laughlin, Barrett-Connor et al. 2000; Hendrix 2005). Recent evidence suggests that early oophorectomy (under age 45) may be associated with earlier mortality (Rocca, Grossardt et al. 2006). The ovaries of postmenopausal women continue to secrete testosterone (Speroff, Glass et al. 1999; Lobo 2001; Hendrix 2005). Post-menopausally, testosterone production is reduced by about 25%, due to a decrease in the source hormone androstenedione.

In both sexes, decline in testosterone levels leads to bone density loss (Jassal, Barrett-Connor et al. 1995; Tenover 1997; Davis and Tran 2001; Lobo 2001) and decline in muscle mass (Lobo 2001; Ellison, Bribiescas et al. 2002). Conversely, high levels of testosterone have been linked to higher risk for prostate cancer (Ellison, Bribiescas et al. 2002). In both sexes, low levels of testosterone have been correlated with lower coital frequency and loss of sexual desire (Davis and Tran 2001). Since testosterone levels decrease gradually over time, the sharp decrease in sexual interest, among menopausal women, does not seem to be solely attributable to declining testosterone levels (Dennerstein, Smith et al. 1994; Davis and Tran 2001). Some studies have shown that testosterone administration improves mood and well-being in both sexes, but especially among men (Jassal, Barrett-Connor et al. 1995; Grinspoon, Corcoran et al. 2000; Ellison, Bribiescas et al. 2002).

## Measurement

Sex hormone assays, particularly in the clinical setting, are typically performed on serum specimens (Kaufman and Lamster 2002). Salivary measures have been developed and offer a relatively convenient and minimally-invasive approach for obtaining sex hormone data (Gallagher, Leitch et al. 2006). Salivary measurements can also be applied as a cost-effective method in a large population setting. (Kaufman and Lamster 2002).

Circulating testosterone in women provides an important biomarker of ovarian androgen production, though approximately one-third of these circulating levels are derived via precursors from the adrenal gland (Lobo 2001). While the salivary levels of testosterone represent only a small percentage of the general circulation levels (Shirtcliff, Granger et al. 2002), salivary testosterone levels in men measured by enzyme immunoassay (EIA) or radioimmunoassay (RIA) are significantly correlated with both free (RIA:  $R^2 = 0.42$ ,  $p < .01$ ; EIA:  $R^2 = 0.29$ ,  $p < .05$ ) and total (RIA:  $R^2 = 0.79$ ,  $p < .001$ ; EIA:  $R^2 = 0.29$ ,  $p < .05$ ) serum levels. In women, salivary levels only significantly correlate with total (RIA:  $R^2 = 0.41$ ,  $p < .01$ ; EIA:  $R^2 = 0.32$ ,  $p < .05$ ) serum levels (Shirtcliff, Granger et al. 2002).

## Population Norms

**Table 1. Testosterone Concentrations**

Group	N	Age	Mean Concentration +/- Dispersion Value	Units	Source
<b>Serum</b>					
Men	1,709	40-70	5.2 SD = +/- 1.8	ng/ml	Feldman, 2002; Massachusetts Male Aging Study
Pre-menopausal women	155	34-83	20-80	ng/dl	Speroff, 1999; Meldrum, Davidson et al., 1981
Pre-menopausal women	15	18-25	44.0 SD = +/- 2.8	ng/dl	Lobo, 2001; Vermeulen, 1976
Perimenopausal women	3,029	mean age: 46.25	46.93 SD = +/- 28.98	ng/dl	Lasley et al., 2002
Perimenopausal women	2,930	42-52	47.0 SD = +/- 30.0 (Median: 42.0)	ng/dl	Randolph et al., 2003
Post-menopausal women	155	34-83	15-70	ng/dl	Speroff, 1999; Meldrum, Davidson et al., 1981
Post-menopausal women	19	51-65	29.7 SD = +/- 4.0	ng/dl	Lobo, 2001; Vermeulen, 1976
Oophorectomized women	8	51-62	12.0 SD = +/- 2.1	ng/dl	Lobo, 2001; Vermeulen, 1976
<b>Saliva*</b>					
Men	26	45-60	238 SEM = +/- 14	pmol/L	Ellison, 2002
Male prison inmates	89	18-23	8.3 SD = +/- 2.9	ng/dl	Dabbs, 1987
Female prison inmates	87	17-60	1.98 SD = +/- 0.61	ng/dl	Dabbs, 1997

\* Few studies report *salivary* testosterone data using a population sample.

<b>Table 2. Testosterone Ranges (in pg/mL)</b>				
	N	Average	Median	Range 5-95%
Females	166	47.62	35.82	8.19-132.23
Males	94	153.43	125.97	53.85-313.41

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Note: These values were taken from young adults, aged 18-30.

## Specimen Collection

All respondents were asked to provide a salivary specimen; 90.8% (N=2,721) agreed. 2,640 respondents were able to provide a salivary specimen. This involved production of approximately 2 milliliters of saliva (unstimulated passive drool) into a small, code-labeled polypropylene collection vial via a 5-centimeter section of a household plastic straw, following procedures recommended by Salimetrics, LLC. The procedure required approximately 5 minutes. The time of last food or water consumption prior to saliva collection was recorded.

## Storage and Shipping

The salivary specimens were transported from the interview to a freezer using cold packs. Salivary specimens were stored in a freezer until they were shipped. The salivary samples were shipped to the lab on dry ice according to instructions. Upon receipt at Salimetrics, specimens were stored at -80°C in lab grade freezers.

<b>Shipping Address</b>	Salimetrics, LLC Attn: Receiving Dept. 101 Innovation Blvd., Suite #302 State College, PA 16803 800-790-2258
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## Assay

(see *Salimetrics Salivary Testosterone Enzyme Immunoassay Kit package insert for details*  
<http://salimetrics.com/pdf/Testo%20Kit%20Insert.pdf> )

On day of assay, the specimens were thawed completely, vortexed, and centrifuged at 1500 x g (@3000 rpm) for 15 minutes. Clear samples were pipetted into wells. The enzyme immunoassay was conducted at Salimetrics, LLC. The assay range was > 1 pg/ml. Assays were conducted in the following priority order: 1) estrogen, 2) progesterone, 3) DHEA, 4) testosterone, 5) cotinine and underwent 2 to 3 freeze-thaw cycles:

*thaw #1: sex hormone assays*

*thaw #2: a subset underwent repeat sex hormone testing based on quality indicators*

*thaw #3: cotinine assay*

**Table 3. NSHAP Salivary Testing Performed at Salimetrics**

Test	Units	Highest Calibrator*	Lowest Calibrator*	Lower limit of sensitivity	None detected (ND) reported if value:	Interference likely if value:
Estradiol	pg/mL	64	2	1 pg/mL	<0.5 pg/mL	>320 pg/mL
Progesterone	pg/mL	2430	10	5 pg/mL	<2 pg/mL	>5x highest calibrator
DHEA	pg/mL	1000	10.2	5 pg/mL	≤2 pg/mL	>5x highest calibrator
Testosterone	pg/mL	600	6.1	1 pg/mL	≤0.5 pg/mL	>5x highest calibrator
Cotinine	ng/mL	200	0.8	0.05 ng/mL	unable to get a number value because result is too low	dilute sample x20; report >3000 if value is still high

\* Calibrator values are used to adjust instrumentation by establishing the relationship (under specified conditions) between known, standard values and the values indicated by a particular measuring instrument. See package insert for calibration curve.

### Scoring

Values reported in picograms per milliliter (pg/mL). Assay range ≥ 1.0 pg/mL.

### Expected Salivary Ranges

**Table 4.**

<i>Adult Males</i>	Midday	70-220* pg/mL
<i>Adult Females</i>	Midday	5-70* pg/mL

\*AM values may be significantly different

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Note: These values were taken from young adults, aged 18-30.

### Performance Characteristics

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#### A. Recovery

Saliva samples containing different levels of endogenous testosterone were spiked with known quantities of testosterone and assayed. The average recovery was 105.0% (range 93.3% to 116.3%), 109.12% for males and 99.6% for females.

#### *B. Intra-assay precision*

Intra-assay precision was determined from the mean of 8 replicates at high (197.3 pg/mL) and low (26.3 pg/mL) testosterone levels. The average intra-assay coefficient of variation was 3.3% and 6.7% for high and low levels.

#### *C. Inter-assay precision*

Inter-assay precision was determined from the mean of averaged duplicates for 10 separate runs at high (200.7 pg/mL) and low (13.1 pg/mL) testosterone levels. The average inter-assay coefficient of variation was 5.1% for high and 9.6% for low testosterone levels.

#### *D. Linearity of dilution*

Saliva samples were diluted (range 1:2 to 1:16) with assay buffer and assayed in duplicate. The average recovery was 101.1% (range 88.2% to 120.2%), 102.86% for males and 98.68% for females.

#### *E. Sensitivity*

The lower limit of sensitivity was determined by interpolating the mean minus 2 SD's for 10 sets of duplicates for the 0 pg/mL standard. The minimal concentration of testosterone that can be distinguished from 0 is <1 pg/mL.

#### *F. Correlation with Serum*

The correlation between saliva and serum free and total testosterone was determined by assaying 32 matched samples (16 adult males and females). The saliva-serum free testosterone correlation (n=30) was,  $r = 0.93$ ,  $p < 0.001$ , and the saliva-serum total testosterone correlation was  $r(30) = 0.929$ ,  $p < 0.001$ . The serum-saliva correlations were stronger for males ( $r = 0.80$  to  $0.85$ ) than for females ( $r = 0.38$  to  $0.48$ ). The conversion equation from salivary concentration to serum concentration for this particular testosterone assay is:

$$\text{[Males] Serum T (ng/mL)} = 0.0401 * \text{salivary T (pg/mL)} + 1.9299$$

$$\text{[Females] Serum T (ng/mL)} = 0.0071 * \text{saliva T (pg/mL)} + 0.7437$$

#### *G. Method Comparison*

The correlation between the Salimetrics EIA and a published serum RIA modified for use with saliva was evaluated by assaying 32 common samples. For the combined sample, the EIA-RIA results (n=30) were highly correlated,  $r = 0.95$ ,  $p < 0.001$ . The EIA-RIA correlation was stronger for males ( $r = 0.88$ ) than for females ( $r = 0.54$ ).

### **Quality Control (see Table 3)**

Run on each EIA test plate were six (6) standard calibrators ranging from 6.1 pg/mL to 600 pg/mL and two sets of high and low controls with established ranges. A sufficient number of assay kits and controls were sequestered for the project to minimize any lot-to-lot variations over the course of the study.

Subjects' saliva samples were run in duplicate (saliva pipetted into side-by-side wells) on a single EIA plate. Assay results for each subject were acceptable when the coefficient of variation (%CV) between the duplicate results (result 1 and result 2) was <15%. In instances where the %CV between duplicates was >15%, results were accepted if the absolute value between result 1 and result 2 was <8 pg/mL. Values greater than the upper assay limit of 600 pg/mL were run on dilution to bring the OD readings within accepted range (6.1 pg/mL - 600 pg/mL). In instances when a sample returned an extremely high result (5 times the highest calibrator), dilutions were made up to 3,000 pg/mL and a flag (\*\*) and comment "interference likely" were added to the report. Samples with results < 1 pg/mL were also repeated. Values falling between 0.51 pg/mL and 6.1 pg/mL were reported and flagged (\*) with the comment "below lower limit of assay". Testosterone values less than or equal to 0.5 pg/mL were reported as "none detected". In some cases the initial values did not fall within the accepted criteria (CV% <15% or difference <8 pg/mL between values 1 & 2 ) but there was no sample remaining to re-run the assay. These data were reported with the comment "quantity not sufficient (qns) to repeat". In some instances there was no sample remaining after first three assays in sequence (estradiol, progesterone, DHEA) were completed. The test result was then reported as "quantity not sufficient (qns)".

Testosterone data were compiled in Excel by the testing manager and checked for accuracy by the technical supervisor before final reports were emailed. Data was supplied with corresponding assay plate number to facilitate the calculation of intra-assay and inter-assay control values.

### **Availability**

<b>Product Name</b>	Expanded Range (ER) Salivary Testosterone Assay
<b>Manufacturer</b>	Salimetrics LLC
<b>Location of Manufacturer</b>	101 Innovation Blvd., Suite 302 State College, PA 16803 USA 800-790-2258 (USA & Canada only)
<b>Catalog No.</b>	1-2402/1-2412

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