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National Social Life, Health, and Aging Project (NSHAP)

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Technical Report: Salivary Assays

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Cotinine

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

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Rationale

Cotinine is a metabolite of nicotine (Etzel 1990; Jenkins and Counts 1999; Terry, Hernandez et al. 2005). Nicotine is metabolized in the liver, where it undergoes oxidation (Vartiainen, Seppala et al. 2002). On average, 9% of the original nicotine is excreted intact, while 70% is converted into cotinine (Vartiainen, Seppala et al. 2002). Since cotinine has a longer half-life than nicotine (approximately 15-19 hours)(Terry, Hernandez et al. 2005), it is retained in the body for a

considerable period of time (Dhar 2004) . Thus, cotinine can be detected in various body fluids, and is used to assess an individual's exposure to tobacco and/or tobacco smoke (Jenkins and Counts 1999; Ziegler, Kauczok et al. 2004).

Cotinine levels are used to quantify tobacco use and to determine second-hand smoking exposure (Jenkins and Counts 1999; Dhar 2004; Farrelly, Nonnemaker et al. 2005). Some major health consequences of tobacco use and exposure include coronary heart disease (Whincup, Gilg et al. 2004), lung cancer (Boffetta, Clark et al. 2006), and asthma (Mannino, Homa et al. 2002). While self-reports of cigarette smoking are useful, they may not capture exposure to second-hand smoke (Webb 2003; Caraballo, Giovino et al. 2004). Cotinine levels have also been used to validate self-reported smoking data (Vartiainen, Seppala et al. 2002; Sandhu, Humphris et al. 2004).

Measurement

Cotinine can be extracted through a variety of bodily fluids such as urine, serum, and saliva specimens (Etzel 1990; Jenkins and Counts 1999; Dhar 2004), as well as amniotic fluid, cervical mucus, and hair (Etzel 1990). It can then be quantified, through a number of methods, such as enzyme immunoassay (Dhar 2004; Salimetrics 2006), radioimmunoassay, gas-liquid chromatography, and liquid chromatography (Etzel 1990; Dhar 2004).

A saliva-serum correlation of $r = 0.99$ ($n = 567$; mean age = 46.1, range = 18-88 years; 52% male) has been found, where the salivary cotinine level = 1.25 X plasma cotinine level (95% CI = 1.23 -1.26). In addition, the slope of saliva-serum linear regression equation becomes significantly steeper (salivary cotinine level = 1.30 X plasma cotinine level, 95% CI = 1.29-1.32) among older subjects (Jarvis, Primatesta et al. 2003). In another study, a high saliva-serum correlation ($r = 0.84$, $p < 0.001$) was reported among 327 subjects (age range unspecified) (van Vunakis, Tashkin et al. 1989). Highly correlated, both serum and saliva-based assay methods are appropriate for tobacco exposure measurement (van Vunakis, Tashkin et al. 1989; Jarvis, Primatesta et al. 2003; Binnie, McHugh et al. 2004). However, salivary specimen collection is preferred to serum (Binnie, McHugh et al. 2004) or urine (Berny, Boyer et al. 2002), because of its ease of use and minimal invasiveness.

In a recent study examining sex hormone and nicotine metabolism, it was observed that pre-menopausal women processed nicotine and cotinine faster than men, but there was little gender difference when post-menopausal women were compared to similarly-aged men (Benowitz, Lessov-Schlaggar et al. 2006). Although the pharmacokinetics of cotinine do not differ significantly between older adults and middle-aged adults, renal clearance of cotinine decreased by about 20% in elderly adults, due to the diminished glomerular filtration rate associated with aging (Molander, Hansson et al. 2001).

Population Norms

Several studies report population distributions of cotinine (Haley, Axelrad et al. 1983; Jarvis, Russell et al. 1983; Jarvis, Tunstall-Pedoe et al. 1984; Jarvis, Russell et al. 1985; Abrams, Follick et al. 1987; Coultas, Howard et al. 1987; Jarvis, McNeill et al. 1987; Jarvis, Tunstall-Pedoe et al. 1987; Langone, Cook et al. 1988; Istvan, Nides et al. 1994; Etter, Vu Duc et al. 2000; Salimetrics 2006).

Table 1. Salivary Cotinine Concentrations (in ng/ml) Among Non-smokers (no exposure to nicotine)

Author	N	Age	Mean +/- SD	Range (if available)	Assay Method*
Abrams et al.	30	N/A**	0.3 +/- 1.6	0-9	RIA
Coultas et al.	181	18-29	1.6 +/- 2.8		RIA
Etter et al.	97	19-61	6.3 +/- 24.2	0-46	GLC
Jarvis et al. (1987)	330	11-16	1.97		GLC
Jarvis et al. (1983)	7	N/A**	1.5		GLC
Jarvis et al. (1985)	269	11-16	0.44 +/- 0.68		GLC
Jarvis et al. (1984)	46	Mean = 56.8 +/ 10.1	0.73		GLC
Langone et al.	36	4-5	0.81 +/- 3.3	0-19	RIA
Salimetrics	115	Mean = 25.63	0.91 +/- 1.43		EIA
Salimetrics	115	Mean = 7.3 months	2.26 +/- 3.3		EIA

*RIA = radioimmunoassay, GLC = gas-liquid chromatography, EIA = enzyme immunoassay

** Not specified

Table 2. Salivary Cotinine Concentrations (in ng/ml) Among Self-Reported Smokers

Author	N	Age	Mean +/- SD	Range (if available)	Assay Method*
Abrams et al.	63	Mean = 41	348.2 +/- 195.4	26-933	RIA
Coultas et al.	366	N/A**	296 +/- 208		RIA
Etter et al.	207	19-61	166 +/- 170	0-838	GLC
Haley et al.	12	Adult	361 +/- 80		RIA
Istvan et al.	3,538 (men)	35-59	384 +/- 203		RIA
Istvan et al.	2,096 (women)	35-59	354 +/- 189		RIA
Jarvis et al. (1984)	94	N/A**	309.9		GLC
Jarvis et al. (1987)	75	N/A**	330 +/- 190		GLC
Langone et al.	26	N/A**	392 +/- 377	48-2000	RIA
Salimetrics	82	Mean = 24.43	252.86 +/- 179		EIA
Salimetrics	82	Mean = 7.16 months	10.96 +/- 9.08		EIA

*RIA = radioimmunoassay, GLC = gas-liquid chromatography, EIA = enzyme immunoassay

** Not specified

Table 3. Mean plasma and salivary cotinine concentrations, in ng/ml, among smokers and nonsmokers [Mean age for all subjects = 18-88] (Jarvis, Primatesta et al. 2003)

Subgroup	N	Plasma Cotinine	Saliva Cotinine	Ratio (saliva vs. plasma)
All nonsmokers	292	1.41	1.74	1.24
No smokers in household	226	1.09	1.33	1.22
Smoker in household	51	2.83	3.55	1.26
All smokers	275	235.9	295.2	1.25
All subjects	567	115.2	144.2	1.25

Besides inhalation, increases in salivary cotinine levels may also occur through exposure to nicotine by other routes, such as chewing nicotine gum, chewing tobacco, using smokeless tobacco, and snuff dipping. Cotinine concentrations in saliva, among nonsmokers, averaged 0.58% of levels found in smokers (Etzel 1990).

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.

Shipping Address	Salimetrics, LLC Attn: Mary Curran 101 Innovation Blvd., Suite #302 State College, PA 16803 800-790-2258

Assay

(see Salimetrics, LLC Salivary Cotinine Quantitative Immunoassay Kit package insert for details http://salimetrics.com/pdf/Cotinine%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. 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*

*A duplicate cotinine assay (for quality control) was not always performed. If enough sample remained for one assay (singlet), this was performed instead.

Table 4. 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 nanograms per milliliter (ng/mL). Assay range ≥ 0.05 ng/mL.

Performance Characteristics

A. Recovery

Eight saliva samples were spiked with known quantities of cotinine and assayed.

Table 5.

Sample	Endogenous (ng/mL)	Added (ng/mL)	Expected (ng/mL)	Observed (ng/mL)	Recovery (%)
1	0	100	100	87.61	87.6
1	0	10	10	10.64	106.4
2	0	30	30	28.26	94.2
2	0	3.75	3.75	3.47	92.5
3	594.90	30	624.90	602.70	96.4
4	627.95	3.75	631.70	661.95	104.8
5	267.39	30	297.39	290.40	97.6
6	68.46	1.00	69.46	60.11	86.5
7	188.03	500	688.03	612.17	89.0
8	100.44	500	600.44	538.24	89.6

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

B. Precision

The intra-assay precision was determined from 28 samples of high and 12 samples of low levels of cotinine.

Table 6.

Sample	N	Mean (ng/mL)	Std Dev (ng/mL)	Coefficient of Variation (%)
Low	12	5.91	0.13	2.3
High	28	92.67	5.41	5.8

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

The inter-assay precision was determined from the mean of average duplicates for 12 separate runs.

Table 7.

Sample	N	Mean (ng/mL)	Std Dev (ng/mL)	Coefficient of Variation (%)
Low	12	5.09	0.49	8.2
High	12	101.96	5.06	5.0

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

C. Sensitivity

The lower limit of sensitivity was determined by interpolating the mean minus 2 SD's for 20 zero values. The minimal concentration of cotinine that can be distinguished from zero is 0.05 ng/mL.

D. Linearity of Dilution

Two saliva samples were diluted with assay diluent and assayed.

Table 8.

Sample	Dilution Factor	Expected (ng/mL)	Observed (ng/mL)	Recovery (%)
Sample 1			87.61	
	1:2	43.81	43.13	98.4
	1:4	21.90	21.99	100.4
	1:8	10.95	12.30	112.3
	1:16	5.48	6.10	111.3
	1:32	2.73	2.99	109.5
	1:64	1.37	1.50	109.5
Sample 2			586.39	
	1:2	293.20	291.84	99.5
	1:4	146.60	148.91	101.6
	1:8	73.30	73.63	100.5
	1:16	36.65	38.56	105.2
	1:32	18.32	19.14	104.5
	1:64	9.16	10.29	112.3

Reproduced with permission from Cotinine Quantitative Immunoassay Kit, 1-2002/1-2012, 96-Well Kit, April 4, 2006 *(Salimetrics 2006)*

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

E. Measurement of Salivary Cotinine in Smokers and Non-smokers Using the Salimetrics EIA Kit

Table 9.

Group	N	Mean (ng/mL)	Std Dev (ng/mL)	Range (ng/mL)
Adult Smokers	21	206.33	123.47	47.87 - 586.39
Non-smokers	10	0	0	NA

The Salimetrics EIA is able to distinguish smokers from non-smokers with 100% accuracy.
Reproduced with permission from Cotinine Quantitative Immunoassay Kit, 1-2002/1-2012, 96-Well Kit, April 4, 2006 *(Salimetrics 2006)*

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

Quality Control (see Table 4)

Run on each EIA test plate were six (6) standard calibrators ranging from 0.8 ng/mL to 200 ng/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 <1.0 ng/mL. Values greater than the upper assay limit of 200 ng/mL were run on dilution to bring the OD readings within acceptable range. High concentrations of cotinine were diluted up to a final dilution of 1:20. If the OD readings were still out-of-range, a value of >3000 ng/mL was reported. Data < 0.05 ng/mL were reported and flagged (*) with the comment "below lower limit of assay". Results with low values (based on OD reading) but not returning a number value were reported as "none detected (ND)". Every effort was made to return a numerical result. Since this was the last assay performed in the testing sequence every effort was made to return at least one value (singlet). If this was not possible, "quantity not sufficient (qns)" was reported as the result.

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

Availability

Product Name	High Sensitivity Salivary Cotinine Enzyme Immunoassay Kit
Manufacturer	Salimetrics LLC
Location of Manufacturer	101 Innovation Blvd., Suite 302 State College, PA 16803 USA 800-790-2258 (USA & Canada only)
Catalog No.	1-2002/1-2012, 96-Well Kit

References

Abrams, D. B., M. J. Follick, et al. (1987). "Saliva cotinine as a measure of smoking status in field settings." Am J Public Health **77**(7): 846-8.

Benowitz, N. L., C. N. Lessov-Schlaggar, et al. (2006). "Female sex and oral contraceptive use accelerate nicotine metabolism." Clinical Pharmacology & Therapeutics **79**(5): 480-488.

Berny, C., J. C. Boyer, et al. (2002). "[Biomarkers of tobacco smoke exposure]." Ann Biol Clin (Paris) **60**(3): 263-72.

Binnie, V., S. McHugh, et al. (2004). "The validation of self-reported smoking status by analysing cotinine levels in stimulated and unstimulated saliva, serum and urine." Oral Diseases **10**(5): 287-293.

Boffetta, P., S. Clark, et al. (2006). "Serum cotinine level as predictor of lung cancer risk." Cancer Epidemiology Biomarkers & Prevention **15**(6): 1184-1188.

Caraballo, R. S., G. A. Giovino, et al. (2004). "Self-reported cigarette smoking vs. serum cotinine among US adolescents." Nicotine & Tobacco Research **6**(1): 19-+.

Coultas, D. B., C. A. Howard, et al. (1987). "Salivary cotinine levels and involuntary tobacco smoke exposure in children and adults in New Mexico." Am Rev Respir Dis **136**(2): 305-9.

Dhar, P. (2004). "Measuring tobacco smoke exposure: quantifying nicotine/cotinine concentration in biological samples by colorimetry, chromatography and immunoassay methods." Journal of Pharmaceutical and Biomedical Analysis **35**(1): 155-168.

Etter, J. F., T. Vu Duc, et al. (2000). "Saliva cotinine levels in smokers and nonsmokers." Am J Epidemiol **151**(3): 251-8.

Etzel, R. A. (1990). "A Review of the Use of Saliva Cotinine as a Marker of Tobacco-Smoke Exposure." Preventive Medicine **19**(2): 190-197.

Farrelly, M. C., J. M. Nonnemaker, et al. (2005). "Changes in hospitality workers' exposure to secondhand smoke following the implementation of New York's smoke-free law." Tobacco Control **14**(4): 236-241.

Haley, N. J., C. M. Axelrad, et al. (1983). "Validation of self-reported smoking behavior: biochemical analyses of cotinine and thiocyanate." Am J Public Health **73**(10): 1204-7.

Istvan, J. A., M. A. Nides, et al. (1994). "Salivary cotinine, frequency of cigarette smoking, and body mass index: findings at baseline in the Lung Health Study." Am J Epidemiol **139**(6): 628-36.

Jarvis, M., H. Tunstall-Pedoe, et al. (1984). "Biochemical markers of smoke absorption and self reported exposure to passive smoking." J Epidemiol Community Health **38**(4): 335-9.

Jarvis, M. J., A. D. McNeill, et al. (1987). "Passive smoking in adolescents: one-year stability of exposure in the home." Lancet **1**(8545): 1324-5.

Jarvis, M. J., P. Primatesta, et al. (2003). "Measuring nicotine intake in population surveys: Comparability of saliva cotinine and plasma cotinine estimates." Nicotine & Tobacco Research **5**(3): 349-355.

Jarvis, M. J., M. A. Russell, et al. (1983). "Absorption of nicotine and carbon monoxide from passive smoking under natural conditions of exposure." Thorax **38**(11): 829-33.

Jarvis, M. J., M. A. Russell, et al. (1985). "Passive exposure to tobacco smoke: saliva cotinine concentrations in a representative population sample of non-smoking schoolchildren." Br Med J (Clin Res Ed) **291**(6500): 927-9.

Jarvis, M. J., H. Tunstall-Pedoe, et al. (1987). "Comparison of tests used to distinguish smokers from nonsmokers." Am J Public Health **77**(11): 1435-8.

Jenkins, R. A. and R. W. Counts (1999). "Personal exposure to environmental tobacco smoke: salivary cotinine, airborne nicotine, and nonsmoker misclassification." J Expo Anal Environ Epidemiol **9**(4): 352-63.

Langone, J. J., G. Cook, et al. (1988). "Monoclonal antibody ELISA for cotinine in saliva and urine of active and passive smokers." J Immunol Methods **114**(1-2): 73-8.

Mannino, D. M., D. M. Homa, et al. (2002). "Involuntary smoking and asthma severity in children - Data from the Third National Health and Nutrition Examination Survey." Chest **122**(2): 409-415.

Molander, L., A. Hansson, et al. (2001). "Pharmacokinetics of nicotine in healthy elderly people." Clin Pharmacol Ther **69**(1): 57-65.

Salimetrics (2006). High Sensitivity Salivary Cotinine Quantitative Enzyme Immunoassay Kit. State College, PA, Salimetrics LLC**:** 3.

Salimetrics (2006). Salivary Testosterone Enzyme Immunoassay Kit.

Sandhu, S., G. Humphris, et al. (2004). "Smoking habits in patient's who have been treated for an oral cancer: validation of self-report using saliva cotinine." Oral Oncology **40**(6): 576-578.

Terry, A. V., C. M. Hernandez, et al. (2005). "Cotinine, a neuroactive metabolite of nicotine: Potential for treating disorders of impaired cognition." Cns Drug Reviews **11**(3): 229-252.

van Vunakis, H., D. P. Tashkin, et al. (1989). "Relative sensitivity and specificity of salivary and serum cotinine in identifying tobacco-smoking status of self-reported nonsmokers and smokers of tobacco and/or marijuana." Arch Environ Health **44**(1): 53-8.

Vartiainen, E., T. Seppala, et al. (2002). "Validation of self reported smoking by serum cotinine measurement in a community-based study." Journal of Epidemiology and Community Health **56**(3): 167-170.

Webb, D. A., Boyd R. N, et al. (2003). "The discrepancy between self-reported smoking status and Urine cotinine levels among women enrolled in prenatal care at four publicly funded clinical sites." Journal of Public Health Management Practice **9** (4): 322-325.

Whincup, P. H., J. A. Gilg, et al. (2004). "Passive smoking and risk of coronary heart disease and stroke: prospective study with cotinine measurement." British Medical Journal **329**(7459): 200-204.

Ziegler, U. E., J. Kauczok, et al. (2004). "Clinical correlation between the consumption of nicotine and cotinine concentrations in urine and serum by competitive enzyme-linked Immunosorbent assay." Pharmacology **72**(4): 254-259.

Dehydroepiandrosterone (DHEA)

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

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Rationale

The adrenal cortex is the primary source of circulating concentrations of DHEA and DHEA-S, (Williams and Wilson 1998) which are secreted in response to adrenal corticotropin-releasing hormone (ACTH) (Parker, Azziz et al. 1996). DHEA and DHEA-S act as the precursors (Regelson, Loria et al. 1994) to the approximately 50% of androgens in adult men, 75% of active estrogens in premenopausal women, and almost all of active estrogens after menopause (Labrie, Belanger et al. 1997).

DHEA production and concentration declines significantly and steadily during aging (Ravaglia, Forti et al. 1996; Speroff, Glass et al. 1999; Hackbert and Heiman 2002; Villareal and Holloszy 2004). Some have considered DHEA concentration, along with its sulfated counterpart, DHEA-S, to be possible markers of physiologic aging (Rudman, Shetty et al. 1990).

Inverse relationships between DHEA concentration and extent of cardiovascular disease (Alexandersen, Haarbo et al. 1996), progression of HIV infection (Christeff, Lortholary et al. 1996), symptomatic progression of systemic lupus erythematosus (SLE) (Spark 2002), presence of rheumatoid arthritis among postmenopausal women (Masi 1995), and bone resorption and skin pigmentation (Spark 2002) have been reported. Activities, such as smoking, among middle-aged men (Field, Colditz et al. 1994), and exercise (Kroboth, Salek et al. 1999), have been shown to influence DHEA concentration.

DHEA has been explored for its role in mood and well-being (Hackbert and Heiman 2002; Spark 2002), sexual physiology and treatment of sexual problems (Spark 2002).

Measurement

Sex hormone assays, particularly in the clinical setting, are typically performed on a serum specimen. Salivary measures have been developed and offer a relatively convenient and minimally-invasive approach for obtaining sex hormone data (Gallagher, Leitch et al. 2006). These measures are representative of active, unbound steroid concentrations in the blood. No serum binding globulin has been identified for DHEA (Gallagher, Leitch et al. 2006).

DHEA is rapidly cleared from the blood (Longcope 1996) Levels in saliva are significantly lower than levels found in circulating serum (Gallagher, Leitch et al. 2006; Salimetrics 2006). Previous studies have indicated a strong positive correlation between salivary and serum DHEA levels (Shirtcliff, Granger et al. 2001; Gallagher, Leitch et al. 2006), though as expected, absolute concentration of DHEA is higher in serum. In a recent study done by Shirtcliff et al. (2001), the relationship between salivary DHEA levels and serum DHEA was only maintained when samples were collected using non-cotton based methods.

Scoring

Salivary values are reported in picograms per milliliter (pg/mL). The assay range was ≥5 pg/mL. (Serum values are typically reported in nanograms/mL.)

Population Norms

Note: 1 nanogram (ng) = 1,000 picogram (pg)

Table 1.

*Salivary DHEA Expected Ranges:

Group	N	Mean (pg/mL)	Standard Deviation (pg/mL)
Females	19	165.6	71.6
Males	20	153.5	68.8

Reproduced with permission from DHEA Quantitative Immunoassay Kit, 1-1502/1-1512, 96-Well Kit, April 10, 2006 *(Salimetrics 2006)*

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

DHEA concentrations have generally been reported to be higher in women than in men (Sulcova, Hill et al. 1997), though some have reported equal concentrations (Labrie, Belanger et al. 1997). The following are population-based, serum DHEA data, and non-population-based salivary data:

- Massachusetts Male Aging Study (Feldman, Longcope et al. 2002):
  - *Serum* samples were taken from 1,709 men 40 to 70 years of age (mean = 55.2) at baseline (1987-1989). Average DHEA = 2.3 ng/ml, +/- 1.6 (standard deviation). At follow-up (1995-1997), 1,156 samples were taken (mean age = 62.7). Average DHEA = 1.9 +/- 1.0 ng/ml.
- Massachusetts Women's Health Study (Johannes, Stellato et al. 1999):
  - *Serum* samples were taken from 224 women, 50 to 60 years of age (mean = 52.7). Mean DHEA level was 2.19 ng/ml.

Table 2. Salivary Concentrations

Group	N	Age	Mean Concentration +/- Dispersion Value*	Units	Source
Saliva					
Women	16	30-34	374.0 SEM = +/- 35.4	pg/ml	(Granger, Schwartz et al. 1999)
Women	16	35-39	299.4 SEM = +/- 30.9	pg/ml	(Granger, Schwartz et al. 1999)
Women	16	40-45	207.6 SEM = +/- 32.7	pg/ml	(Granger, Schwartz et al. 1999)

* Averaged AM and PM values.

Specimen Collection

All NSHAP 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 polystyrene 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.

Shipping and Storage

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.

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

Assay

(see Salimetrics Salivary DHEA Enzyme Immunoassay Kit package insert for details (http://salimetrics.com/pdf/DHEA%20Kit%20Insert.pdf) (Salimetrics 2006))

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 > 5 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 ≥ 5 pg/mL.

Performance Characteristics

A. Precision

Intra-assay precision was determined from the mean of 12 replicates at high (618.6 pg/mL) and low (44.6 pg/mL) DHEA levels. The average intra-assay coefficient of variation was 5.6%.

Inter-assay precision was determined from the mean of averaged duplicates for 12 separate runs. The average inter-assay coefficient of variation was 7.9% for high (579.5 pg/mL) and 8.5% for low (34.8 pg/mL) DHEA levels.

B. Sensitivity

The lower limit of sensitivity was determined by interpolating the mean minus 2 SD for 10 sets of duplicates for the 0 pg/mL standard. The minimal concentration of DHEA that can be distinguished from 0 is 5 pg/mL.

C. Expected Ranges

Table 4.

*Salivary DHEA Expected Ranges:

Group	N	Mean (pg/mL)	Standard Deviation (pg/mL)
Females	19	165.6	71.6
Males	20	153.5	68.8

Reproduced with permission from DHEA Quantitative Immunoassay Kit, 1-1502/1-1512, 96-Well Kit, April 10, 2006 *(Salimetrics 2006)*

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

D. Correlation with Serum

The DHEA serum-saliva correlation, using a log 10 transformation for the total (n = 39), combined males and females, is 0.857, $p < 0.0001$ (Salimetrics 2006). The conversion equation from salivary concentration to serum concentration for this particular DHEA assay is:

Serum DHEA (ng/mL) = 0.0576*saliva DHEA (pg/mL) + 2.3074.

Quality Control (see Table 3)

Run on each EIA test plate were six (6) standard calibrators ranging from 10.2 pg/mL to 1000 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 1000 pg/mL were run on dilution to bring the OD readings within accepted range (10.2 pg/mL - 1000 pg/mL). In instances when a sample returned an extremely high result (5 times the highest calibrator), dilutions were made up to 5,000 pg/mL and a flag (**) and comment "interference likely" were added to the report. Samples with results < 5 pg/mL were also repeated. Values falling between 2.1 pg/mL and 5 pg/mL were reported and flagged (*) with the comment "below lower limit of assay." DHEA values less than or equal to 2 pg/mL were reported as "none detected". In rare instances, repeat results where lower than initial values and did not fall within the accepted criteria (CV% <15% or difference <8 pg/mL between values 1 & 2). These data were reported with the comment "repeat affected by freeze/thaw." If not enough sample remained to run a duplicate assay, "quantity not sufficient (qns)" was reported as the result.

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

Availability

Product Name	Salivary DHEA Enzyme Immunoassay Kit
Manufacturer	Salimetrics, LLC
Location of Manufacturer	101 Innovation Blvd., Suite 302 State College, PA 16803 USA 800-790-2258 (USA & Canada only)
Catalog No.	1-1202/1-1212, 96-Well Kit

References

Alexandersen, P., J. Haarbo, et al. (1996). "The relationship of natural androgens to coronary heart disease in males: A review." Atherosclerosis **125**(1): 1-13.

Christeff, N., O. Lortholary, et al. (1996). "Relationship between sex steroid hormone levels and CD4 lymphocytes in HIV infected men." Experimental and Clinical Endocrinology & Diabetes **104**(2): 130-136.

Feldman, H. A., C. Longcope, et al. (2002). "Age trends in the level of serum testosterone and other hormones in middle-aged men: Longitudinal results from the Massachusetts Male Aging Study." Journal of Clinical Endocrinology and Metabolism **87**(2): 589-598.

Field, A. E., G. A. Colditz, et al. (1994). "The Relation of Smoking, Age, Relative Weight, and Dietary-Intake to Serum Adrenal-Steroids, Sex-Hormones, and Sex Hormone-Binding Globulin in Middle-Aged Men." Journal of Clinical Endocrinology and Metabolism **79**(5): 1310-1316.

Gallagher, P., M. M. Leitch, et al. (2006). "Assessing cortisol and dehydroepiandrosterone (DHEA) in saliva: effects of collection method." J Psychopharmacol **20**(5): 643-9.

Granger, D. A., E. B. Schwartz, et al. (1999). "Assessing dehydroepiandrosterone in saliva: a simple radioimmunoassay for use in studies of children, adolescents and adults." Psychoneuroendocrinology **24**(5): 567-579.

Hackbert, L. and J. R. Heiman (2002). "Acute dehydroepiandrosterone (DHEA) effects on sexual arousal in postmenopausal women." J Womens Health Gend Based Med **11**(2): 155-62.

Johannes, C. B., R. K. Stellato, et al. (1999). "Relation of dehydroepiandrosterone and dehydroepiandrosterone sulfate with cardiovascular disease risk factors in women: Longitudinal results from the Massachusetts women's health study." Journal of Clinical Epidemiology **52**(2): 95-103.

Kroboth, P. D., F. S. Salek, et al. (1999). "DHEA and DHEA-S: A review." Journal of Clinical Pharmacology **39**(4): 327-348.

Labrie, F., A. Belanger, et al. (1997). "Marked decline in serum concentrations of adrenal C19 sex steroid precursors and conjugated androgen metabolites during aging." Journal of Clinical Endocrinology and Metabolism **82**(8): 2396-2402.

Longcope, C. (1996). "Dehydroepiandrosterone metabolism." Journal of Endocrinology **150**: S125-S127.

Masi, A. T. (1995). "Sex-Hormones and Rheumatoid-Arthritis - Cause or Effect Relationships in a Complex Pathophysiology." Clinical and Experimental Rheumatology **13**(2): 227-240.

Parker, C. R., R. Azziz, et al. (1996). "Adrenal androgen production in response to adrenocorticotropin infusions in men." Endocrine Research **22**(4): 717-722.

Ravaglia, G., P. Forti, et al. (1996). "The relationship of dehydroepiandrosterone sulfate (DHEAS) to endocrine-metabolic parameters and functional status in the oldest-old. Results from an Italian study on healthy free-living over-ninety-year-olds." Journal of Clinical Endocrinology and Metabolism **81**(3): 1173-1178.

Regelson, W., R. Loria, et al. (1994). "Dehydroepiandrosterone (Dhea) - the Mother Steroid .1. Immunological Action." Aging Clock **719**: 553-563.

Rudman, D., K. R. Shetty, et al. (1990). "Plasma dehydroepiandrosterone sulfate in nursing home men." J Am Geriatr Soc **38**(4): 421-7.

Salimetrics (2006). Salivary DHEA Enzyme Immunoassay Kit. State College, PA Salimetrics LLC**:** 3.

Salimetrics (2006). Salivary Testosterone Enzyme Immunoassay Kit.

Shirtcliff, E. A., D. A. Granger, et al. (2001). "Use of salivary biomarkers in biobehavioral research: cotton-based sample collection methods can interfere with salivary immunoassay results." Psychoneuroendocrinology **26**(2): 165-73.

Spark, R. F. (2002). "Dehydroepiandrosterone: a springboard hormone for female sexuality." Fertil Steril **77 Suppl 4**: S19-25.

Speroff, L., R. H. Glass, et al. (1999). Clinical gynecologic endocrinology and infertility. Philadelphia, Lippincott Williams & Wilkins.

Sulcova, J., M. Hill, et al. (1997). "Age and sex related differences in serum levels of unconjugated dehydroepiandrosterone and its sulphate in normal subjects." J Endocrinol **154**(1): 57-62.

Villareal, D. T. and J. O. Holloszy (2004). "Effect of DHEA on abdominal fat and insulin action in elderly women and men - A randomized controlled trial." Jama-Journal of the American Medical Association **292**(18): 2243-2248.

Williams, R. H. and J. D. Wilson (1998). Williams textbook of endocrinology. Philadelphia, Saunders.

Estradiol

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

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Rationale

Estradiol (17-beta-estradiol or E_2), a steroid hormone derived from cholesterol, targets a variety of tissues, located in the female and male reproductive tracts, mammary gland, and skeletal and cardiovascular systems (Hall, Couse et al. 2001). Among women, it is primarily synthesized from testosterone in the ovarian follicles, whereas among men, it is produced by the testes and extraglandular conversion of androgens (Tivis, Richardson et al. 2005; Salimetrics 2006).

In women, estradiol synthesis normally declines after menopause (Manly, Merchant et al. 2000; Meston and Frohlich 2000). This causes decreased vaginal lubrication and atrophy of the vaginal epithelium, due to diminished genital vasocongestion (Meston and Frohlich 2000). Though the role of estrogens in female sexual desire is not fully understood, they may influence sexual desire (Dennerstein, Gotts et al. 1994; Meston and Frohlich 2000); estrogen replacement therapy may indirectly enhance female sexuality, by restoring vaginal lubrication (Meston and Frohlich 2000) or promoting positive body image and overall sense of well-being.

A number of studies have linked higher levels of serum estradiol to increased risk for developing breast cancer (Clemons and Goss 2001; Chlebowski, Hendrix et al. 2003; Tivis, Richardson et al. 2005) and coronary problems (Manson, Hsia et al. 2003; Tivis, Richardson et al. 2005). For women, higher estradiol levels have also been associated with improved cognition (Maki and Resnick 2000; Carlson, Zandi et al. 2001; Tivis, Richardson et al. 2005), mood, and memory (Tivis, Richardson et al. 2005). Estradiol's effects on women's skeletal health, however, are inconclusive.

For elderly men, estradiol may have a role, in combination with testosterone and other factors, in preservation of memory and cognitive function (Barrett-Connor, Goodman-Gruen et al. 1999; Carlson and Sherwin 2000).

Measurement

Sex hormone assays, particularly in the clinical setting, are typically performed on a serum specimen (Kaufman and Lamster 2002). Salivary measures have been developed and offer a relatively convenient and minimally-invasive approach for obtaining sex hormone data (Worthman, Stallings et al. 1990; Kaufman and Lamster 2002; Granger, Shirtcliff et al. 2004). These measures are representative of active, unbound steroid concentrations in the blood (Worthman, Stallings et al. 1990; Lu, Bentley et al. 1999).

In the case of estradiol, good correlation between salivary and serum levels have been reported (Worthman, Stallings et al. 1990), including one study of people ages 18 to 28 (Shirtcliff, Granger et al. 2000) ($n = 31$) finding a correlation of $r = 0.68$ ($p < 0.001$), where a stronger, significant association was detected among women ($n = 16$, $r = 0.60$, $p < 0.013$) than men ($n = 15$, $r = 0.60$, $p>0.05$). Reported salivary concentrations, relative to free serum concentrations, have varied (Worthman, Stallings et al. 1990) from 0.2% to 7.90% (Lu, Bentley et al. 1999). In one study, salivary estradiol concentrations accounted for, on average, 1.65% of serum concentrations among men, and 3.33% among women (Shirtcliff, Granger et al. 2000). Among premenopausal women, salivary estradiol levels vary significantly across the menstrual cycle (Shirtcliff, Granger et al. 2000), with the lowest levels occurring during menstruation (Shirtcliff, Granger et al. 2000; Salimetrics 2006).

Population Norms

To our knowledge, population-based, published literature on *salivary* estradiol levels, particularly for postmenopausal women and older men, are limited.

Table 1. Mean Serum and Salivary Estradiol Levels

	Age Range	N	Mean SERUM levels (pg/ml)	SD	Range	Mean SALIVARY levels (pg/ml)	SD	Range	Source
Male	*8-9*	17				0.83		< 0.25 - 2.46	*[1]*
	18-27	15	32.37	7.53		0.53	0.19		*[1]*
	32-49	19				1.02		< 0.25 - 3.93	*[1]*
Female	*11-12 (noncycling)*	18				0.82		< 0.25 - 2.45	*[1]*
	18-27	16	70.39	45.42		0.98	0.56		*[1]*
	36-51 (cycling)	54				2.01		< 0.25 - 6.13	*[1]*
	48-65 (w/ estrogen therapy)	28	136.17*	17.76	55.1 - 496.3	1.61	0.23	0.36 - 6.17	*[2]*
	50-66 (postmenopausal)	12	15.48	2.29	4.0 - 28.3	1.39	0.33	0.54 - 4.78	*[2]*
	Perimenopausal women, mean age: 46.25 (SD = 2.63)	3,029	77.3	81.1					*[3]*
	42-52 (perimenopausal women)	2,930	76.0 (Median: 54.0)	77.0					*[4]*

*Women who were undergoing estrogen therapy had a significantly higher serum estradiol level ($p < 0.0001$)
[1] Shirtcliff et al. 2000
[2] Tivis et al. 2005
[3] Lasley et al. 2002
[4] Randolph et al. 2003

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.

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

Assay

(see Salimetrics Salivary Estradiol Enzyme Immunoassay Kit package insert for details http://salimetrics.com/pdf/HS%20Estradiol%20E2%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 2. 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 pg/mL.

Performance Characteristics

A. Precision

Table 3.

The intra-assay precision was determined from the mean of 14 replicates each.

Sample	N	Mean (pg/ml)	Standard Deviation (pg/ml)	Coefficient of Variation (%)
High	14	20.26	1.42	7.0
Mid	14	7.24	0.45	6.3
Low	14	3.81	0.31	8.1

The inter-assay precision was determined from the mean of average duplicates for 10 separate runs.

Sample	N	Mean (pg/ml)	Standard Deviation (pg/ml)	Coefficient of Variation (pg/ml)
High	10	24.62	1.47	6.0
Low	10	4.76	0.42	8.9

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B. Sensitivity

The lower limit of sensitivity was determined by interpolating the mean minus 2 SD's of the optical densities of 10 sets of duplicates at the 0 pg/mL standard. The minimal concentration of estradiol that can be distinguished from 0 is less than 1.0 pg/mL.

C. Correlation with Serum

The correlation between saliva and serum estradiol in females was determined by assaying 11 matched samples. Samples were screened for pH and blood contamination. The magnitude of the saliva-serum correlation, r= 0.80, p <0.001, is consistent with the literature, in that the saliva-serum correlation is stronger among women, than men (Shirtcliff, Granger et al. 2000), and intra-subject correlation is higher, compared to between-subjects (Ellison and Lipson 1999). The conversion equation from salivary concentration to serum concentration for this particular estradiol assay is:

Serum E2 (pg/mL) = 2.171*salivary E2 (pg/mL) +7.1846.

D. Specificity of Antiserum

Table 4.

Compound	Spiked Concentration (ng/mL)	% Cross-reactivity in HS Salivary Estradiol EIA
Estriol	10	0.234
Estrone	1	1.276
Progesterone	100	ND
17 α-Hydroxyprogesterone	1000	ND
Testosterone	1000	ND
Cortisol	1000	ND
DHEA	1000	ND
Androstenedione	1000	ND
Aldosterone	1000	ND
Cortisone	1000	ND
11-Deoxycortisol	1000	ND
21-Deoxycortisol	1000	ND
Dexamethasone	1000	ND
Triamincinolone	1000	ND
Corticosterone	1000	ND
Prednisolone	1000	ND
Prednisone	100	0.016
Transferrin	1000	ND

ND = None detected (<0.004)

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E. Recovery

Table 5.

Five saliva samples was spiked with different levels of estradiol and assayed.

Sample	Endogenous (pg/ml)	Added (pg/ml)	Expected (pg/ml)	Observed (pg/ml)	Recovery (%)
I	2.92	20.48	23.40	23.84	101.9
II	4.68	13.65	18.33	17.91	97.7
III	3.80	3.20	7.00	6.78	96.9
IV	5.41	20.48	25.89	28.2	108.9
V	3.69	3.20	7.16	8.26	115.4

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F. Linearity of Dilution

Table 6.

Four saliva samples were diluted with PBS and assayed.

Sample	Dilution	Expected (pg/ml)	Observed (pg/ml)	Recovery (%)
I			28.98	
	1:2	14.49	13.57	93.7
	1:4	7.25	7.24	99.9
	1:8	3.62	3.73	103.0
II			23.84	
	1:2	11.92	12.03	100.9
	1:4	5.96	5.56	93.3
	1:8	2.98	3.60	120.8
III			6.78	
	1:2	3.39	3.07	90.6
	1:4	1.70	1.70	100.0
IV			8.54	
	1:2	4.27	4.55	106.6
	1:4	2.14	1.93	90.2

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Quality Control (see Table 2)

Run on each EIA test plate were six (6) standard calibrators ranging from 2 pg/mL to 64 pg/mL and two sets of high and low controls. The control ranges were established using a minimum of 10 sets of data points across 10 plates. The mean and standard deviations were calculated and a range was established (mean +/- 2 S.D.). 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 values: 1.) fell within the range of the calibrators (2 pg/mL - 64 pg/mL) , 2.) were >1 pg/mL, and 3.) 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 <2 pg/mL. Values greater than the upper

assay limit of 64 pg/mL were run on dilution to bring the OD readings within acceptable range (2 pg/mL - 64 pg/mL). In instances when a sample returned an extremely high result, dilutions were made up to 320 pg/mL and a flag (**) and comment “interference likely” were added to the report. Samples with results < 1pg/mL were also repeated. Values falling between 0.5 pg/mL and 1 pg/mL were reported and flagged (*) with the comment “below lower limit of assay”. Estradiol values less than 0.5 pg/mL were reported as “none detected”.

Estradiol 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

Product Name	High Sensitivity Salivary Estradiol Enzyme Immunoassay Kit
Manufacturer	Salimetrics LLC
Location of Manufacturer	101 Innovation Blvd., Suite 302 State College, PA 16803 USA 800-790-2258 (USA & Canada only)
Catalog No.	1-3702/1-3712, 96-Well Kit

References

Barrett-Connor, E., D. Goodman-Gruen, et al. (1999). "Endogenous sex hormones and cognitive function in older men." J Clin Endocrinol Metab **84**(10): 3681-5.

Carlson, L. E. and B. B. Sherwin (2000). "Higher levels of plasma estradiol and testosterone in healthy elderly men compared with age-matched women may protect aspects of explicit memory." Menopause **7**(3): 168-77.

Carlson, M. C., P. P. Zandi, et al. (2001). "Hormone replacement therapy and reduced cognitive decline in older women: the Cache County Study." Neurology **57**(12): 2210-6.

Chlebowski, R. T., S. L. Hendrix, et al. (2003). "Influence of estrogen plus progestin on breast cancer and mammography in healthy postmenopausal women: the Women's Health Initiative Randomized Trial." Jama **289**(24): 3243-53.

Clemons, M. and P. Goss (2001). "Estrogen and the risk of breast cancer." N Engl J Med **344**(4): 276-85.

Dennerstein, L., G. Gotts, et al. (1994). "The relationship between the menstrual cycle and female sexual interest in women with PMS complaints and volunteers." Psychoneuroendocrinology **19**(3): 293-304.

Ellison, P. T. and S. F. Lipson (1999). "Salivary estradiol--a viable alternative?" Fertil Steril **72**(5): 951-2.

Granger, D. A., E. A. Shirtcliff, et al. (2004). "The "trouble" with salivary testosterone." Psychoneuroendocrinology **29**(10): 1229-40.

Hall, J. M., J. F. Couse, et al. (2001). "The multifaceted mechanisms of estradiol and estrogen receptor signaling." J Biol Chem **276**(40): 36869-72.

Kaufman, E. and I. B. Lamster (2002). "The diagnostic applications of saliva--a review." Crit Rev Oral Biol Med **13**(2): 197-212.

Lasley BL, Santoro N, Randolf JF, Gold EB, Crawford S, Weiss G, et al. The relationship of circulating dehydroepiandrosterone, testosterone, and estradiol to stages of the menopausal transition and ethnicity. Journal of Clinical Endocrinology & Metabolism 2002;87(8):3760-7.

Lu, Y., G. R. Bentley, et al. (1999). "Salivary estradiol and progesterone levels in conception and nonconception cycles in women: evaluation of a new assay for salivary estradiol." Fertil Steril **71**(5): 863-8.

Maki, P. M. and S. M. Resnick (2000). "Longitudinal effects of estrogen replacement therapy on PET cerebral blood flow and cognition." Neurobiol Aging **21**(2): 373-83.

Manly, J. J., C. A. Merchant, et al. (2000). "Endogenous estrogen levels and Alzheimer's disease among postmenopausal women." Neurology **54**(4): 833-7.

Manson, J. E., J. Hsia, et al. (2003). "Estrogen plus progestin and the risk of coronary heart disease." N Engl J Med **349**(6): 523-34.

Meston, C. M. and P. F. Frohlich (2000). "The neurobiology of sexual function." Arch Gen Psychiatry **57**(11): 1012-30.

Randolph, J. F., Jr., M. Sowers, et al. (2003). "Reproductive hormones in the early menopausal transition: relationship to ethnicity, body size, and menopausal status." Journal of Clinical Endocrinology & Metabolism **88**(4): 1516-22.

Salimetrics (2006). High Sensitivity Salivary Estradiol Enzyme Immunoassay Kit. State College, PA, Salimetrics LLC**:** 3.

Salimetrics (2006). Salivary Testosterone Enzyme Immunoassay Kit.

Shirtcliff, E. A., D. A. Granger, et al. (2000). "Assessing estradiol in biobehavioral studies using saliva and blood spots: simple radioimmunoassay protocols, reliability, and comparative validity." Horm Behav **38**(2): 137-47.

Tivis, L. J., M. D. Richardson, et al. (2005). "Saliva versus serum estradiol: implications for research studies using postmenopausal women." Prog Neuropsychopharmacol Biol Psychiatry **29**(5): 727-32.

Worthman, C. M., J. F. Stallings, et al. (1990). "Sensitive salivary estradiol assay for monitoring ovarian function." Clin Chem **36**(10): 1769-73.

Progesterone

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

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Date: December 17, 2007

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Rationale

Derived from cholesterol, progesterone is a steroid hormone, found in both females and males (Andersen and Tufik 2006). In females, progesterone is produced in the adrenal cortex, corpus

luteum and, during pregnancy, the placenta. In reproductive age females, the primary function of progesterone involves preparation and maintenance of the endometrium for implantation of fertilized eggs (Speroff, Glass et al. 1999). Progesterone levels are used to assess ovarian function and defects, as well as monitoring of infertility treatments (Groschl, Rauh et al. 2001; Ishikawa, Sengoku et al. 2002).

In postmenopausal women, there is a significant decrease in progesterone levels. Because progesterone may be linked to sexuality, this decline may be related to diminished sexual function and desire (Dennerstein, Alexander et al. 2003). Increased levels of progesterone have also been found in states of stress and anxiety; this may relate to its sedative or stress-counteracting effects (Wirth and Schultheiss 2006; Wirth, Meier et al. 2007). Pharmaceutical doses of progesterone have been used to decrease sexual desire in men, however the natural, physiological role of progesterone in men has not been extensively researched (Andersen and Tufik 2006).

Measurement

Sex hormone assays, particularly in the clinical setting, are typically performed on a serum specimen (Kaufman and Lamster 2002). Salivary measures have been developed and offer a relatively convenient and minimally-invasive approach for obtaining sex hormone data (Worthman, Stallings et al. 1990; Kaufman and Lamster 2002; Granger, Shirtcliff et al. 2004). These measures are representative of active, unbound steroid concentrations in the blood (Worthman, Stallings et al. 1990; Lu, Bentley et al. 1999).

Salivary and serum progesterone concentrations are highly correlated (Bolaji 1994; Ellison and Lipson 1999; Lu, Bentley et al. 1999), In a study by Lu et al. (1997) among women aged 20-40 (n = 48), serum-saliva progesterone correlation, in the luteal phase of menstrual cycle, was reported to be highly correlated ($r = 0.75$ ($P < 0.001$)) (Lu, Chatterton et al. 1997). Reported salivary concentrations, in proportion to free serum concentrations, have varied from 0.82% to 2.1%. In earlier work summarized by Lu et al., saliva-serum correlations were also high: ($r = 0.75$ and 0.93) (Lu, Bentley et al. 1999). Among pre-menopausal women, a substantial rise in salivary progesterone has been reported on the day after ovulation (Lu, Bentley et al. 1999).

Materials used in the construction of collection tubes and vials (Lu, Chatterton et al. 1997) and blood contamination (Kivlighan, Granger et al. 2005) may affect salivary progesterone measurements.

Population Norms

To our knowledge, no population-based data on serum progesterone levels are available.

Table 1. Salivary Progesterone Levels in Women During Different Phases of Menstrual Cycle

Group	N	Mean (pg/mL)	SD (pg/mL)
Follicular phase*	127	80.35	34.8
Luteal phase*	202	131.00	54.5
Pre-menopausal, day 20*	23	136.30	82.3
Post menopausal, day 20**	11	58.90	29.7

Reproduced with permission from Progesterone Quantitative Immunoassay Kit, 1-1502/1-1512, 96-Well Kit, April 10, 2006 *(Salimetrics 2006)*

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

** These values were taken from subjects aged 50-70.

Young Adults

Table 2. Mean Salivary Progesterone Levels Measured Among Women During Different Menstrual Phases and Among Men (in pg/mL) (SEM)						
	N	Mean Age	Premenstrual Phase	Midcycle Phase	Menstrual Phase	Source
Normal-cycling women	11	22.5	88.0 (13.1)	26.4 (2.1)	27.9 (2.7)	(Schultheiss, Dargel et al. 2003)
Normal-cycling women	38	19.78	-	38 (6)	-	(Schultheiss, Wirth et al. 2004)
Women taking oral contraceptives	12	21	21.1 (1.9)	25.1 (1.8)	21.5 (1.8)	(Schultheiss, Dargel et al. 2003)
Men	12	23	22.8 (1.9)	22.6 (1.3)	24.6 (2.2)	(Schultheiss, Dargel et al. 2003)
Men	20	19.78	-	25 (1)	-	(Schultheiss, Wirth et al. 2004)

Premenopausal Women

Table 3. Salivary Progesterone Levels Measured During Luteal Phase of Premenopausal Women (Mean age 42, N = 38) (Chatterton, Mateo et al. 2005)	
	pmol/L (SEM)
Cycle 1	329 (39)
Cycle 2	517 (43)
Cycle 3	462 (81)
Mean	436 (34)

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.

Shipping and Storage

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.

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

Assay

(see Salimetrics Salivary Progesterone Enzyme Immunoassay Kit package insert for details http://salimetrics.com/pdf/Progesterone%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 4. 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 ≥ 5 pg/mL.

Performance Characteristics

A. Precision

Table 5.

The intra-assay precision was determined from the mean of 12 replicates each.

Sample	N	Mean (pg/mL)	Standard Deviation (pg/mL)	Coefficient of Variation (%)
H	12	884.61	35.1	4.0
L	12	39.23	3.3	8.4

The inter-assay precision was determined from the mean of average duplicates for 12 separate runs.

Sample	N	Mean (pg/mL)	Standard Deviation (pg/mL)	Coefficient of Variation (%)
H	12	884.15	48.7	5.5
L	12	28.04	2.7	9.6

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

B. Sensitivity

The lower limit of sensitivity was determined by interpolating the mean minus 2 SD's for 20 zero standards. The minimal concentration of progesterone that can be distinguished from 0 is 5 pg/mL.

C. Correlation with Serum

The correlation between serum and saliva progesterone was determined by assaying matched samples using the DSL serum progesterone EIA and the Salimetrics HS Salivary Progesterone EIA. The correlation between serum and saliva was highly significant with r = 0.80, (n = 35). For women (n =25), r = 0.87, and for men (n = 8), r = 0.67. The conversion equation from salivary concentration to serum concentration for this particular progesterone assay is:

Serum P (ng/mL) = 0.1387*saliva P (pg/mL) + 0.2097.

D. Specificity of Antiserum

Table 6.

Compound	Spiked Concentration (ng/mL)	% Cross-reactivity
Prednisolone	1000	0.0021
Prednisone	1000	0.0038
Cortisone	1000	0.0106
11-Deoxycortisol	1000	0.0195
21-Deoxycortisol	1000	0.0082
17-α Hydroxy-progesterone	1000	0.0723
Dexamethasone	1000	0.0014
Triamincinolone	1000	ND
Corticosterone	500	0.1924
Testosterone	1000	ND
DHEA	1000	ND
Cortisol	1000	ND
Transferrin	1000	ND
Aldosterone	1000	ND
Estradiol	1000	ND
Estrone	1000	ND
Estriol	1000	ND

ND = None detected (<0.004)

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

Quality Control (see Table 4)

Run on each EIA test plate were six (6) standard calibrators ranging from 10 pg/mL to 2430 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 <10 pg/mL. Values greater than the upper assay limit of 2430 pg/mL were run on dilution to bring the OD readings within accepted range (10 pg/mL - 2430 pg/mL). In instances when a sample returned an extremely high result (5 times the highest calibrator), dilutions were made up to 12,150 pg/mL and a flag (**) and comment "interference likely" were added to the report. Samples with results < 5 pg/mL were also repeated. Values falling between 2 pg/mL and 5 pg/mL were reported and flagged (*) with the comment "below lower limit of assay". Progesterone values less than 2 pg/mL were reported as "none detected". In rare instances, repeat results where lower than initial values and did not fall within the accepted criteria (CV% <15% or difference <10 pg/mL between values 1 & 2). These data were reported with the comment "repeat affected by freeze/thaw".

Progesterone 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

Product Name	Salivary Progesterone Enzyme Immunoassay Kit
Manufacturer	Salimetrics LLC
Location of Manufacturer	101 Innovation Blvd., Suite 302 State College, PA 16803 USA 800-790-2258 (USA & Canada only)
Catalog No.	1-1502/1-1512, 96-Well Kit

References

Andersen, M. L. and S. Tufik (2006). "Does male sexual behavior require progesterone?" Brain Research - Brain Research Reviews **51**(1): 136-43.

Bolaji, II (1994). "Sero-salivary progesterone correlation." International Journal of Gynaecology & Obstetrics **45**(2): 125-31.

Chatterton, R. T., Jr., E. T. Mateo, et al. (2005). "Characteristics of salivary profiles of oestradiol and progesterone in premenopausal women." Journal of Endocrinology **186**(1): 77-84.

Dennerstein, L., J. L. Alexander, et al. (2003). "The menopause and sexual functioning: a review of the population-based studies." Annu Rev Sex Res **14**: 64-82.

Ellison, P. T. and S. F. Lipson (1999). "Salivary estradiol--a viable alternative?" Fertil Steril **72**(5): 951-2.

Granger, D. A., E. A. Shirtcliff, et al. (2004). "The "trouble" with salivary testosterone." Psychoneuroendocrinology **29**(10): 1229-40.

Groschl, M., M. Rauh, et al. (2001). "Relationship between salivary progesterone, 17-hydroxyprogesterone, and cortisol levels throughout the normal menstrual cycle of healthy postmenarcheal girls." Fertility & Sterility **76**(3): 615-7.

Ishikawa, M., K. Sengoku, et al. (2002). "The clinical usefulness of salivary progesterone measurement for the evaluation of the corpus luteum function." Gynecologic & Obstetric Investigation **53**(1): 32-7.

Kaufman, E. and I. B. Lamster (2002). "The diagnostic applications of saliva--a review." Crit Rev Oral Biol Med **13**(2): 197-212.

Kivlighan, K. T., D. A. Granger, et al. (2005). "Blood contamination and the measurement of salivary progesterone and estradiol." Hormones & Behavior **47**(3): 367-70.

Lu, Y., G. R. Bentley, et al. (1999). "Salivary estradiol and progesterone levels in conception and nonconception cycles in women: evaluation of a new assay for salivary estradiol.[see comment]." Fertility & Sterility **71**(5): 863-8.

Lu, Y. C., R. T. Chatterton, Jr., et al. (1997). "Direct radioimmunoassay of progesterone in saliva." J Immunoassay **18**(2): 149-63.

Salimetrics. (2006, April 10, 2006). "Salivary Testosterone Enzyme Immunoassay Kit."

Schultheiss, O. C., A. Dargel, et al. (2003). "Implicit motives and gonadal steroid hormones: effects of menstrual cycle phase, oral contraceptive use, and relationship status." Horm Behav **43**(2): 293-301.

Schultheiss, O. C., M. M. Wirth, et al. (2004). "Effects of affiliation and power motivation arousal on salivary progesterone and testosterone." Hormones & Behavior **46**(5): 592-9.

Speroff, L., R. Glass, et al. (1999). Clinical Gynecologic Endocrinology and Infertility Lippincott Williams & Wilkins.

Wirth, M. M., E. A. Meier, et al. (2007). "Relationship between salivary cortisol and progesterone levels in humans." Biological Pyschology **74**: 104-107.

Wirth, M. M. and O. C. Schultheiss (2006). "Effects of affiliation arousal (hope of closeness) and affiliation stress (fear of rejection) on progesterone and cortisol." Hormones & Behavior **50**(5): 786-95.

Worthman, C. M., J. F. Stallings, et al. (1990). "Sensitive salivary estradiol assay for monitoring ovarian function." Clin Chem **36**(10): 1769-73.

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
Serum					
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
Saliva*					
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.

Table 2. Testosterone Ranges (in pg/mL)

	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

Reproduced with permission from Estradiol Quantitative Immunoassay Kit 1-1402/1-1412, 96-Well Kit, April 10, 2006 *(Salimetrics 2006)*

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.

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

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:
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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.

Adult Males	Midday	70-220* pg/mL
Adult Females	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:

[Males] Serum T (ng/mL) = 0.0401*salivary T (pg/mL) +1.9299

[Females] Serum T (ng/mL) = 0.0071*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

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

References

Davis, S. and J. Tran (2001). "What are "normal" testosterone levels for women?" J Clin Endocrinol Metab **86**(4): 1842-4.

Davis, S. R. and J. Tran (2001). "Testosterone influences libido and well being in women." Trends Endocrinol Metab **12**(1): 33-7.

Dennerstein, L., A. M. Smith, et al. (1994). "Sexuality and the menopause." J Psychosom Obstet Gynaecol **15**(1): 59-66.

Ellison, P. T., R. G. Bribiescas, et al. (2002). "Population variation in age-related decline in male salivary testosterone." Hum Reprod **17**(12): 3251-3.

Feldman, H. A., C. Longcope, et al. (2002). "Age trends in the level of serum testosterone and other hormones in middle-aged men: longitudinal results from the Massachusetts male aging study." J Clin Endocrinol Metab **87**(2): 589-98.

Gallagher, P., M. M. Leitch, et al. (2006). "Assessing cortisol and dehydroepiandrosterone (DHEA) in saliva: effects of collection method." J Psychopharmacol **20**(5): 643-9.

Grinspoon, S., C. Corcoran, et al. (2000). "Effects of hypogonadism and testosterone administration on depression indices in HIV-infected men." J Clin Endocrinol Metab **85**(1): 60-5.

Guyton, A. C. (1991). Textbook of medical physiology. Philadelphia, Saunders.

Hendrix, S. L. (2005). "Bilateral oophorectomy and premature menopause." Am J Med **118 Suppl 12B**: 131-5.

Jassal, S. K., E. Barrett-Connor, et al. (1995). "Low bioavailable testosterone levels predict future height loss in postmenopausal women." J Bone Miner Res **10**(4): 650-4.

Kaufman, E. and I. B. Lamster (2002). "The diagnostic applications of saliva--a review." Crit Rev Oral Biol Med **13**(2): 197-212.

Lasley BL, Santoro N, Randolf JF, Gold EB, Crawford S, Weiss G, et al. The relationship of circulating dehydroepiandrosterone, testosterone, and estradiol to stages of the menopausal transition and ethnicity. Journal of Clinical Endocrinology & Metabolism 2002;87(8):3760-7.

Laughlin, G. A., E. Barrett-Connor, et al. (2000). "Hysterectomy, oophorectomy, and endogenous sex hormone levels in older women: the Rancho Bernardo Study." J Clin Endocrinol Metab **85**(2): 645-51.

Lobo, R. A. (2001). "Androgens in postmenopausal women: production, possible role, and replacement options." Obstet Gynecol Surv **56**(6): 361-76.

Meldrum, D. R., B. J. Davidson, et al. (1981). "Changes in circulating steroids with aging in postmenopausal women." Obstet Gynecol **57**(5): 624-8.

Randolph, J. F., Jr., M. Sowers, et al. (2003). "Reproductive hormones in the early menopausal transition: relationship to ethnicity, body size, and menopausal status." Journal of Clinical Endocrinology & Metabolism **88**(4): 1516-22.

Rocca, W. A., B. R. Grossardt, et al. (2006). "Survival patterns after oophorectomy in premenopausal women: a population-based cohort study." Lancet Oncol **7**(10): 821-8.

Salimetrics. (2006, April 10, 2006). "Salivary Testosterone Enzyme Immunoassay Kit."

Shirtcliff, E. A., D. A. Granger, et al. (2002). "Gender differences in the validity of testosterone measured in saliva by immunoassay." Horm Behav **42**(1): 62-9.

Speroff, L., R. H. Glass, et al. (1999). Clinical gynecologic endocrinology and infertility. Philadelphia, Lippincott Williams & Wilkins.

Tenover, J. L. (1997). "Testosterone and the aging male." J Androl **18**(2): 103-6.

Vermeulen, A. (1976). "The hormonal activity of the postmenopausal ovary." J Clin Endocrinol Metab **42**(2): 247-53.