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

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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.
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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 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

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