



SALIVA BIBLIOGRAPHY

GENERAL

Human Saliva as a Diagnostic Specimen.

Hoffman LF, Journal of Nutrition 13, 2001: 1621S-1625S

Human saliva can be easily obtained by noninvasive techniques and contains many analytes of interest for screening, diagnosis and monitoring. These include steroid and other nonpeptide hormones, therapeutic drugs, drugs of abuse and antibodies. Numerous studies in the past 40 years have shown correlations between serum and saliva levels. Both diurnal and monthly profiles of hormone levels parallel traditional serum patterns. Multiple specimens for steroid hormone analysis can be easily collected by the patient, at home, to monitor fertility cycles, menopausal fluctuations, stress and other diurnal variations. Drug doses can be monitored without inconvenient and costly visits to blood-drawing facilities. Antibody levels can be determined to screen for infectious diseases. Saliva can be collected directly by spitting into a tube or with one of several devices, each of which has its own special advantages and disadvantages. Salivary levels of steroid hormones and other analytes that are protein bound in serum reflect the unbound and active concentration of the hormone. Saliva can be used as a diagnostic specimen not only to obtain information more inexpensively and efficiently than serum, but also to provide information not readily available from serum testing.

The Diagnostic Uses of Saliva.

Mandel ID, Journal of Oral Pathology and Medicine 19(3), 1990: 119-125

It is becoming increasingly apparent to investigators and clinicians in a variety of disciplines that saliva has many diagnostic uses and is especially valuable in the young, the old and infirm, and in large scale screening and epidemiologic studies. The highly sensitive test procedures that are now commonplace makes it practical to quantitate, despite very low concentrations, a large number of hormones and drugs in saliva. Indeed, all steroids of diagnostic significance in routine clinical endocrinology can now be readily measured in saliva. Drug monitoring can include abusive as well as therapeutic agents. The concordance between anti HIV antibodies in saliva and serum has stimulated application

to various other antiviral antibodies, as well as to viral antigens per se. Saliva has found use as a diagnostic aid in an increasing number of clinical situations and in systemic diseases that can affect salivary gland function and composition such as Sjogren's syndrome, cystic fibrosis, and diseases of the adrenal cortex.

The list keeps growing.

The Measurement of Hormones in Saliva: Possibilities and Pitfalls.

Vining RF, McGinley RA. Journal of Steroid Biochemistry 27(1-3), 1987: 81-94

The easy stress-free, non-invasive nature of saliva collection makes it one of the most accessible body fluids and it is potentially of value in studying normal human physiology as well as pathology. Measurements of salivary hormone levels will usually only be of value if they reflect the plasma level of the hormone and the relationship between the saliva and plasma levels of many hormones have been studied by a number of groups. The measurement of the salivary level is a valuable clinical tool for some hormones (e.g. cortisol, oestriol, progesterone), is of little value for others (e.g. cortisone, dehydroepiandrosterone sulphate, thyroxine, pituitary hormones) and for many others the saliva/plasma relationship is not yet sufficiently understood to assess the value of the salivary measurement. As well as reviewing the state of our knowledge of the salivary concentration of many hormones this review outlines a number of "rules of thumb" concerning the presence of hormones in saliva, their saliva/plasma relationship and the potential usefulness of assays of their salivary concentration.

Saliva as a Medium for Investigating Intra- and Interindividual Differences in Sex Hormone Levels in Premenopausal Women.

Gann PH., Giovanazzi S, Van Horn L, Branning A and Chatterton, Jr RT. Cancer Epidemiology Biomarkers & Prevention 10, 2001: 59-64

Repeated measurement of ovarian steroids in saliva could provide an advantage in studies estimating long-term sex steroid exposure in premenopausal women, by reducing the measurement error associated with collection of serum or urine samples. We previously

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reported on characteristics of ultrasensitive RIAs adapted for extraction-free measurement of estradiol (E₂) and progesterone (PG) in saliva. The purpose of the present study was to evaluate the consistency of E₂ and PG levels in saliva in the same women across menstrual cycles, and to compare this with the variation observed between women. We also evaluated the effect of altering the number of consecutive daily samples considered and the method for locating a particular cycle day in relation to ovulation (day 0). Study participants included 12 healthy women who provided daily saliva samples for two consecutive, ovulatory menstrual cycles. A single midluteal serum sample was collected 7–8 days after detection of a luteinizing hormone (LH) peak in urine. We plotted individual cycle profiles and computed intraclass correlation coefficients (ICC) for various definitions of peak and cumulative daily hormone level. For peak PG, determined as the maximal running 3-day mean, ICC was 0.68. For cumulative PG, based on 8 consecutive cycle days (+2 to +9), ICCs were 0.72–0.76 when reverse dating LH peak or rise in salivary PG determined day 0. For E₂, ICCs ranged from 0.74 to 0.79 by various dating methods for the 5 preovulatory days (-4–0), and from 0.85 to 0.92 for the 15 days about the center of the cycle (-6 to +8). With exclusion of just the first 5 days of the cycle, the ICC for E₂ was 0.91. For both E₂ and PG, selection of 5 or 7 days for the estimation of the midluteal mean level provided separation of within and between subject variance that was comparable with a LH-timed serum sample. These results indicate that daily saliva samples can be combined to clarify the interindividual differences in E₂ and PG levels in premenopausal women, and that these interindividual differences may be greater than previously imagined.

Salivary Steroid Assays for Assessing Variation in Endocrine Activity.

Riad Fahmy D, Read GF and Walker RE *J Steroid Biochem* 19, 1982: 265-272

Salivary sampling regimens are non invasive, and therefore facilitate dynamic tests of hormone function and assessment of biological rhythms. Concentrations of neutral steroids in saliva are independent of flow rate and appear to reflect the non protein bound, free(fraction. Comparison of replicate determinations of quality control pools with determinations of samples collected at 2 min intervals allows the significance of short term fluctuations in cortisol and testosterone secretory activity to be estimated. Samples collected at 15 min intervals provide a convenient way to estimate circadian rhythms, particularly in young children. Determination of salivary progesterone concentrations in samples collected by women daily, over extended periods of time, provides a valuable means of assessing ovarian function. Such assays may be used to monitor ovulation induction therapy.

Salivary Testosterone is Associated with Higher Lumbar Bone Mass in Premenopausal Healthy Women with Normal Levels of Serum Testosterone.

Orozco P, Navarro MA and Nolla JM. *European Journal of Epidemiology* 16(10), 2000: 907-912

The relationships among lumbar and femoral bone mineral density (BMD) and different forms of testosterone -total, salivary testosterone and free testosterone index (FTI) calculated with the sex hormone binding globulin (SHBG)-, body mass index (BMI) and body fat distribution (waist-to-hip ratio and breast-to-hip ratio) were analysed in a cross-sectional study with 66 Spanish premenopausal healthy women aged 42 years and with normal levels of serum testosterone. BMD was measured using dual-energy X-ray absorptiometry (Hologic QDR 1000[®]), and salivary and blood samples were obtained during early follicular phase. In a multiple stepwise regression analysis, lumbar BMD was positively predicted by salivary testosterone and negatively by SHBG adjusted by BMI (R² = 0.20; p < 0.02). The most femoral BMDs were negatively predicted by SHBG and positively by breast-to-hip ratio (R² = 0.22–0.33, according to the site measured), but neck BMD was not predicted by any variable. When FTI was entered into the regression model instead of SHBG, it was not an independent predictor of BMD. The waist-to-hip ratio was positively correlated with several femoral BMD sites, but breast-to-hip ratio was better predictor. After adjusting by SHBG, the BMI was only predictor for intertrochanter BMD. All women with elevated salivary testosterone (n = 12) had higher lumbar BMD than those with normal value (1.120 ± 0.112 vs. 1.026 ± 0.118; p < 0.01) without differences in other confounding variables. As a conclusion, in premenopausal healthy women of the same age with normal levels of serum testosterone, low levels of SHBG and high levels of salivary testosterone are associated with higher lumbar BMD, whereas low levels of SHBG together with higher breast-to-hip ratio are associated with higher femoral BMD.

Hormones in Saliva: Mode of Entry and Consequent Implication for Clinical Interpretation.

Vining RE, McGinley RA and Symon RG *Clin Chem* 29, 1983: 1752-1756

Assay of hormones in saliva would be more convenient than asst' in blood, but there is no information on the route by which hormones enter saliva, information that would provide insight into the clinical value of such assays. We have examined the mode of entry of various hormones into saliva. The results suggest that unconjugated steroid enter saliva by diffusing through the cells of the salivary glands and that their concentration in saliva does not depend on the rate of saliva production.

PROGESTERONE

Salivary Progesterone Excellently Reflects Fm and Total Progesterone in Plasma During Pregnancy.

Meulenber PM and Hoffman JA. *Clin Chem* 35, 1989: 168-172

To see if saliva is a valid substitute for plasma in assay of progesterone even when concentrations of hormone and binding proteins are fluctuating, we determined the concentrations of total and free progesterone in plasma and salivary progesterone in specimens from 36 women volunteers during the course of pregnancy and six weeks postpartum, using a highly specific RIA for total progesterone after extraction and chromatographic purification of the steroid. The free fraction in plasma was determined via equilibrium dialysis, followed by the same RIA analysis for progesterone in the dialysate. Despite the dramatic increases in concentrations of total progesterone and binding proteins in plasma during pregnancy, we found highly significant correlations between total and free progesterone in plasma and salivary progesterone in the group as a whole as well as individuals (P<0.001 in almost all cases). The proportion of free progesterone in plasma and of salivary progesterone relative to total progesterone in plasma remained constant at 1% and 0.5%, respectively, whereas during the postpartum period there was much more variance. Evidently salivary progesterone is a very good alternative to plasma as a sample for use in follow up during pregnancy.

Daily Measurements of Salivary Progesterone Reveal a High Rate of Anovulation in Healthy Students.

Vuorento T, Lahti A, Hovatta O, and Huhtaniemi I. *Scared J Clin Lab Invest* 49, 1989: 395 X01

Daily concentrations of salivary progesterone (P) were measured from 32 women during a complete menstrual cycle. Seventeen of the subjects were university students and 15 were patients of an infertility clinic (a severe male factor was verified as the cause of infertility in all of them). Commercially available reagents for radio immunoassay of serum P were modified for salivary measurements, to yield acceptable precision and sensitivity (40 pmol/l). Good correlation (r+0.93) was found between salivary and serum P concentrations in samples collected simultaneously. The follicular phase levels of salivary P were below 100 pmol/l, and those at the luteal peak were 390145 pmol/l (mean±SEM, n=24). From the menstrual salivary P concentration curves we identified the first day of significant elevation above mean follicular levels (T2) and thereafter calculated the cumulative sum of daily P concentrations until 95% of the luteal phase secretion had accumulated (C95). The time needed to reach C95 (designated T95) and logC95 were plotted in coordinates

and used as the basis of evaluation of normal menstrual P secretion. The observations were distributed in two groups, one with clearly identifiable T2 and a distinct luteal phase P (ovulation had occurred) and one with no identifiable T2 and absent luteal phase P peak (indicative of anovulation). Interestingly, 47% of the student population had an abnormally low menstrual P profile while all the other subjects displayed a dear luteal phase peak of salivary P. These data provide more evidence for applicability of salivary P measurements for diagnosis of corpus luteum function and highlight the difficulty of selecting representative reference populations in studies on female reproductive endocrinology.

Longitudinal Studies of Luteal Function by Salivary Progesterone Determinations.

Walker SM, Walker RF and Riad Fahmy D. *Horm Res* 20, 1984: 231-240

A "normal range" for salivary progesterone concentrations has been established using data derived from women who were menstruating regularly and in whom dating of the cycle by accepted criteria was possible. Since these values are in agreement with those in the first 9 days of conception cycles and with those in cycles in which ovulation was confirmed by ultrasonography, they provide a reliable index of progesterone output compatible with fertility. Measurement of daily salivary progesterone values in subfertile women for time spans exceeding 3 months allowed accurate assessment of base line ovarian activity and of the response to ovulation induction therapy. Salivary sampling, by allowing collection of frequent samples with a minimum of time, stress and inconvenience, is ideally suited to longitudinal studies of ovarian activity. This sampling regimen is also applicable to the monitoring of progesterone output throughout pregnancy.

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ESTRADIOL

Sensitive Salivary Estradiol Assay for Monitoring Ovarian Function.

Worthman CM, Stallings JF, Hofman L. *Clin Chem* 36, 1990: 1769-1773

Measurement of steroids in saliva has excited interest because of the numerous potential clinical applications; non invasive, convenient sampling; and apparently accurate reflection of the concentrations of physiologically active unbound steroid in the circulation. Although assays of saliva for several steroid hormones are available and widely used, assays for salivary estradiol are not, primarily because of methodological limitations. By modifying a commercially available kit for serum estradiol, our laboratory has developed a procedure that is sensitive, highly specific, and reliable for measuring salivary estradiol. Assay sensitivity is 0.58 fmol (0.14 pg; sample concentration 1.3 pmol/L) with a mean interassay CV of 10.8% at low concentrations. Clinical studies showed that values for serum and saliva are highly correlated ($P < 0.001$), and demonstrated reliable detection of estradiol peaks during normal ovulatory cycles in serial samples from 15 women. Salivary estradiol peaked at 5.4 (SD 1.9) pmol/L on cycle day 14.4 (SD 3.2), 1.2 (SD 0.8) days before ovulation detected by ultrasound. This assay may be particularly helpful in investigating ovarian function and free estradiol in women at various stages of the reproductive cycle.

Direct Chemiluminescence Immunoassay of Estradiol in Saliva.

DeBoever J, Kohen F, Bouve J, Leysele D and Vanderkerckhove D. *Clin Chem* 36: 2036 2041, 1990

A sensitive and simple direct solid phase chemiluminescence immunoassay is described for estradiol in saliva. In this assay, a second antibody is bound to the wells of microtiter plates. Either buffer with standards or saliva (100 μ L) is incubated in these wells with monoclonal anti estradiol antibody and with estradiol isoluminol conjugate. Incubation time is 2 h. Chemiluminescence of the bound fraction is measured in a manually operated luminometer (Biocounter). The assay has a detection limit of 3.8 pmol/L; analytical recovery of added estradiol is 96.8 \pm 6 (SD 7.0%); within and between assay CVs range between 2.5 \pm 6 and 12.7 \pm 6. Forty unknown saliva samples can be assayed and results calculated within 4.5 h. Results of a slightly modified procedure with black microtiter plates and a prototype of an automated plate reader (Luminoskan) compare well with those of the described method ($r=0.97$). Because steroid binding globulins have been found in saliva, the effect of displacing agents on the results of the direct chemiluminescence assay is described.

TESTOSTERONE

Salivary Testosterone Measurements: Reliability Across Hours, Days, and Weeks.

Dabbs JM Jr. *Physiology and Behavior* 48, 1990: 83-86

Salivary testosterone measurements would appear to be useful in behavioral research, where subjects are often reluctant to provide serum samples. The usefulness of salivary measurements depends upon their reliability, however, which was the focus of the present investigation. In four studies, 270 male and 175 female subjects collected saliva samples at times ranging from 30 min to 8 weeks apart. Subjects collected samples on at least two days, at time of awakening, midmorning, late afternoon, and late evening. Mean reliability was $r=0.64$ across two days and $r=0.52$ across seven eight weeks. Menstrual cycle effects were negligible. Reliability can be increased by using more than one measurement, and it is probably desirable to combine measurements taken several weeks apart. Salivary assays offer a practical way of measuring testosterone in free ranging subjects outside the laboratory.

Salivary Testosterone Measurements: Collecting, Storing, and Mailing Saliva Samples.

Dabbs JM Jr. *Physiology and Behavior* 49, 1991: 815-817

Salivary testosterone measurements can be especially useful in field studies, but reliable ways of collecting and handling samples need to be established. Using cotton dental rolls to collect saliva leads to inflated testosterone scores. Sugar free gum can be used satisfactorily to stimulate saliva among both male and female subjects. Leaving unpreserved saliva samples at room temperature for 2 weeks or mailing them unrefrigerated is satisfactory for male subjects but leads to inflated scores for female subjects.

Salivary Testosterone in Men: Further Evidence of a Direct Correlation with Free Serum Testosterone.

Wang C, Plymate S, Nieschlag E, et al. *J Clin Endocrinol Metab* 53, 1981: 1021-1024

An excellent correlation was found between salivary testosterone (T) and serum T concentrations, as measured by RIA. Using polyacrylamide gel electrophoresis, we have demonstrated that sex steroid-globulin could not be identified in the saliva of men with serum sex steroid binding globulin. After exogenous T administration, saliva and serum T rose abruptly and in parallel. Salivary T concentrations in male patients with thyrotoxicosis were similar to those in normal males, whereas the serum T and sex steroid binding globulin values were significantly higher in the hyperthyroid patients. This study demonstrates that salivary T levels may be used as an index of free serum T.

Menstrual Variation in Salivary Testosterone Among Regularly Cycling Women.

Campbell BC and Ellison PT *Horm Res* 37, 1992: 132-136

To determine menstrual variation in salivary testosterone daily saliva samples were collected from 20 regularly cycling women. Results indicate that the menstrual profile of salivary testosterone for both ovulatory and anovulatory cycles exhibits local peaks during the follicular phase and at midcycle, as well as a luteal trough. However, the testosterone profile for anovulatory cycles exhibited a luteal midcycle peak than that for ovulatory cycles, as well as significantly higher average testosterone levels. These results extend the observation of a midcycle peak in serum testosterone to saliva and suggest the existence of a follicular peak in unbound testosterone coincident with the early androgen production of a cohort of developing follicles.

Bioavailable Testosterone in Salivary Glands.

Pardridge Wand Demers LM. *Clin Chem* 37, 1991: 139-140

The determination of testosterone in the clinical evaluation of hyper and hypoandrogenic states has historically involved the measurement of total and free testosterone in the circulation. The free testosterone has been determined as the absolute free concentration, which normally represents only 2-3% of the total testosterone. Testosterone circulates bound primarily to sex hormone binding globulin (SHBG) and albumin, with a only a very small and insignificant fraction bound to cortisol binding globulin (CBG). Under normal circumstances, 44% of the testosterone in the male is SHBG bound, while 54% is bound to albumin. In females, the figures are 78% SHBG bound and 20% albumin bound. In recent years the concept of bioavailable testosterone has emerged, in which the testosterone fraction bound to albumin is considered to be as readily available to the tissues as is absolute free testosterone; the non SHBG bound fraction, which includes both the free and albumin bound fraction, appears to represent the biologically active form of the hormone. A few laboratories have also used the term free and weakly bound! to indicate the non SHBG bound fraction as the free fraction along with the total testosterone concentration.

Recent evidence suggests that steroid measurements in saliva may be a useful, noninvasive approach to the assessment of free hormone concentrations. The salivary concentrate represents a filtered fraction of the hormone and is independent of flow rate. The study by Swinkels et al. (1) in this issue addresses the bioavailable testosterone concept in saliva and examines the issue of non SHBG bound testosterone vs absolute free testosterone in this context.

Comparison of the Testosterone to Cortisol Ratio Values Obtained from Hormonal Assays in Saliva and Serum.

Obminski Z. *J Sports Med Phys Fitness* 37, 1997: 50-55

Testosterone (T) and cortisol (C) were determined in serum and saliva, sampled simultaneously, from triathletes and karate athletes, in order to determine the T:C ratios in these body fluids and the relationship between them, as well as to assess the salivary T:C ratio as a measure of the so called anabolic catabolic index. Mean salivary T:C (value 1.6710.85) was nearly 3 fold lower than that obtained for serum (4.8711.86). Salivary and serum values were strongly correlated with one another ($r=0.874$, $P < 0.001$) but the relationship depended on the range of cortisol concentrations in serum, the slope of the saliva serum regression line being significantly lower for serum cortisol concentrations over 600 nmol/l than for concentrations below that value (0.305 and 0.380, $P < 0.05$, respectively). It has been concluded that the salivary T:C ratio, based on values reflecting the levels of biologically active fractions of T and C in circulation, is a better measure of metabolic equilibrium conditioned by those hormones that the corresponding ratio obtained from total concentrations in serum.

The Determination of Bio-available Testosterone.

Wheeler MJ. *Ann Clin Biochem* 37, 1997: 50-55

Salivary testosterone determination in studies of child health and development.

Granger DA, Schwartz EB, Booth A, Arentz M. *Hormones and Behavior* 35, 1999:18-27.

ESTRIOL

Relation of Estriol in Saliva to Serum Estriol During Normal Pregnancy.

Rischer Rasmussen W, Gabrielsen MV and Wisborg T Acta Obstet Gynecol 60, 1982: 417-420

As saliva is an easily accessible biological material, compared with 24 hour urine and blood, the salivary concentration of estriol was studied from the 30th to the 41st week of gestation in 268 samples from 124 normal pregnancies. At the same time, venous blood samples were drawn and analyzed for total and unconjugated estriol. The mean values for the concentration of total estriol in saliva in the 30th and 41st weeks were 2.8 and 7.2 nmol/l respectively. The salivary estriol concentration appears to increase in perfect conformity with its serum concentration in the course of gestation. If estriol in saliva also reflects low serum values and an impaired function of the fetoplacental unit, analyses of the saliva may be applicable as a screening procedure in high risk pregnancies.

Saliva as a Fluid for Measurement of Estriol Levels.

Voss HE Biex, Inc., 6693 Sierra Ln, Suite F, Dublin, CA 94568, 1999

As a diagnostic fluid for determining estriol level, saliva offers numerous advantages, most of which relate to its reliability and the noninvasive nature of its collection. Besides lacking the pathos of blood, saliva is an accessible fluid that can easily be collected by the patient. In fact, it is quickly gaining prominence as the matrix of choice for the monitoring of steroid hormones and numerous pharmaceuticals. The collection procedure is neither painful nor traumatic, and the specimen requires no time consuming serum separation step. Moreover, the sample contains biologically active hormone. As a result both the collection and measurement of saliva samples are considerably less expensive than with other commonly used biologic matrices.

CORTISOL

Salivary Cortisol Assays for Assessing Pituitary Adrenal Reserve.

Peters JR, Walker RF, Riad Fahmy D and Hall R Clin Endocrinol 17, 1982: 583-592

Cortisol concentrations were determined in matched samples of plasma and saliva from patients and healthy volunteers throughout the course of standard tests of pituitary and adrenal reserve. During insulin tolerance

tests the percentage incremental changes in cortisol concentrations in saliva were strictly comparable with those in plasma and showed less inter subject variance. The clinical decision taken with regard to the integrity of the pituitary adrenal axis was the same whether plasma or salivary cortisol was measured. In the short tetracosactrin test changes in salivary cortisol reflected those in plasma and patients with loss of adrenal responsiveness would have been diagnosed as such using either measurement. In normal subjects, the circadian rhythm in salivary cortisol concentrations exactly paralleled that in plasma. Absence of the circadian rhythm in cases of hypercortisolism was seen as well in saliva as in plasma. Assays for salivary cortisol therefore provide information which is as clinically useful as that of plasma determinations. Since salivary cortisol concentrations were shown to reflect the five, biologically active fraction in plasma, salivary assay may, in selected cases, provide results of greater diagnostic significance than plasma total concentrations.

Salivary Cortisol: A Practical Method for Evaluation of Adrenal Function.

Kahn JP, Rubinow DR, Davis CL, Kling M and Post RM. Biol Psychiatry 23, 1988: 335-349

Salivary cortisol represents a simple, noninvasive, stress free measure that can greatly facilitate the longitudinal study of hypothalamic pituitary adrenal axis activity in patients with psychiatric disorders. By means of a slight modification of a commercially available radioimmunoassay kit, we studied the stability of salivary cortisol under different conditions, as well as the relationship between plasma and salivary cortisol under basal circadian conditions and following stimulation (CRH) and suppression (dexamethasone). We observed that salivary cortisol was quite stable at room temperature without centrifugation and that salivary and plasma cortisol values were highly correlated. Additionally, we observed a close correspondence in circadian and ultradian fluctuations in salivary and plasma cortisol. The salivary cortisol response to ovine and human CRH was similar to that observed with plasma cortisol, but was greater in magnitude. Finally, employing a plasma criterion as the standard, salivary measures identified 48% of the nonsuppressed Dexamethasone Suppression Tests (DSTs) and 97% of the suppressed DSTs.

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