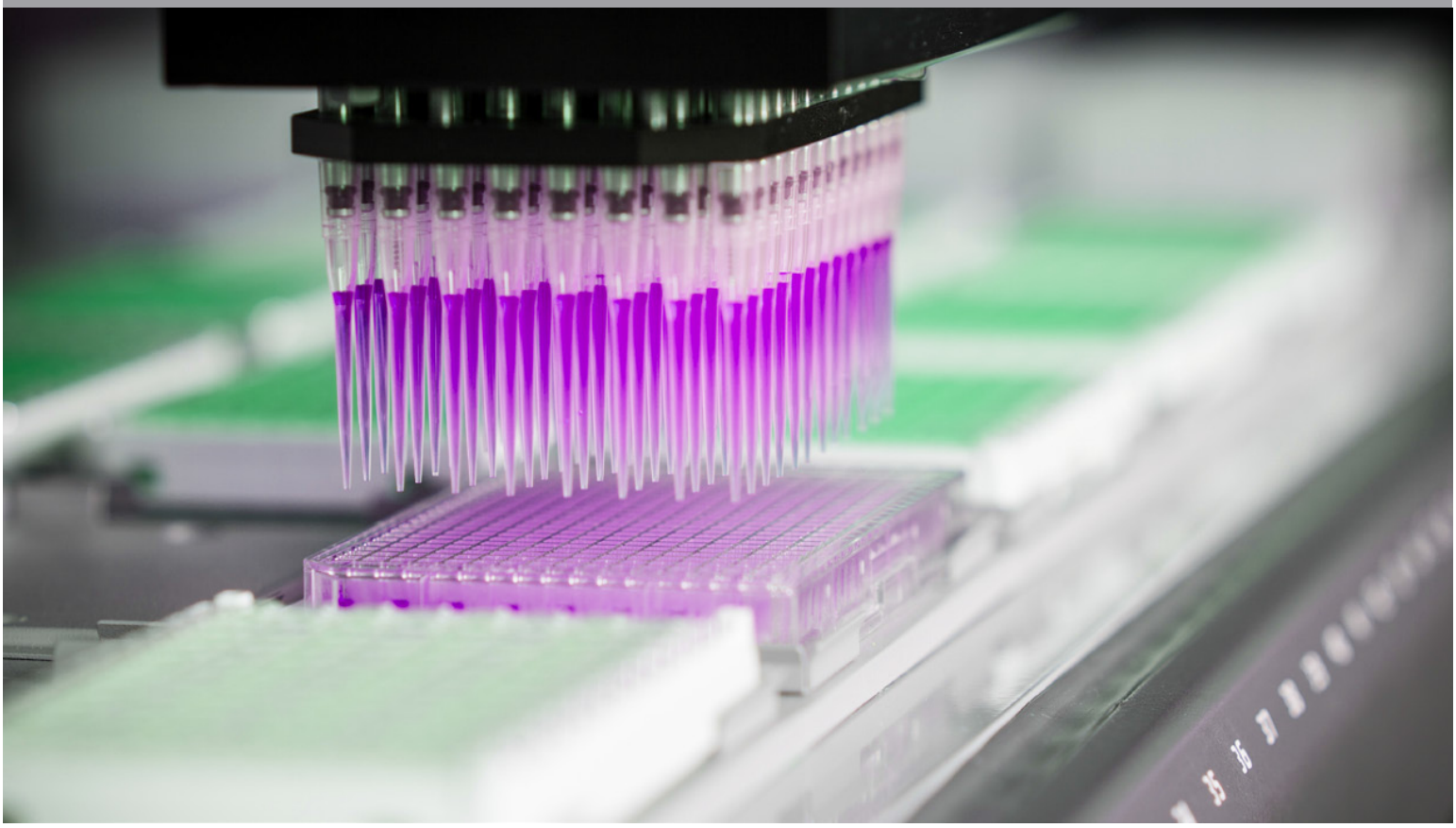
SALIVA TESTING

Scientific Evidence of Testing Accuracy



Saliva testing has been scientifically established as a highly accurate medium for measuring hormone levels equivalent to conventional blood testing. In fact, in 2012 alone over 600 hundred studies were published in peer-reviewed journals using saliva analysis of hormones. Enclosed is an overview of the accuracy and relevance of salivary testing for clinical purposes.

Revised 12.04.13

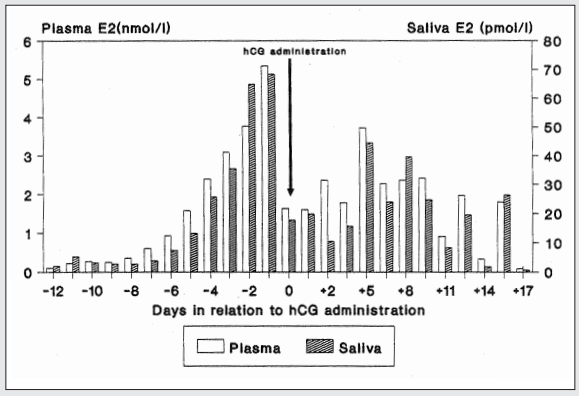
ZRT LABORATORY

Studies Have Found Excellent Correlations Between Saliva & Traditional Serum Assays

The exceptional parallel between saliva and serum has been established over a multitude of different study objectives, health topics and patient demographics. Studies demonstrate that the meaningful equivalencies between saliva and serum testing methodologies are more than sufficient to ensure accuracy and clinical relevance of saliva test results.

Findings of note include:

- Saliva testosterone levels shows excellent correlation to free serum testosterone in men[1] (see graph at right)
- Estradiol and progesterone saliva/serum correlations are consistently observed throughout pre- and post-menopausal concentration ranges[2]
- Saliva estradiol closely compares with plasma levels during menstrual cycle[3] (see graph below)



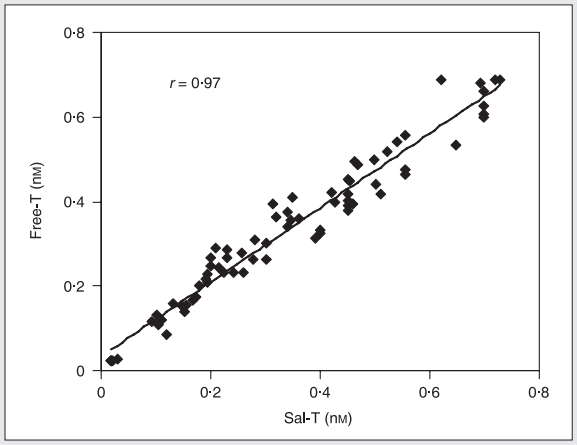
Plasma and salivary E2 in a study of ovulation induction.

Reproduced with permission from assesment of Hormones and Drugs in Saliva in Biobehavioral Research by Clemens Kirschbaum et al., ISB 0-88937-050-8, page 278. Copyright 1992 by Hogrefe & Huber Publishers.

- Significant compatibility is observed between both total and free plasma progesterone and saliva progesterone[4]
- Late night salivary cortisol shows excellent reproducibility within individuals, demonstrating the accuracy of this methodology[5]
- Saliva estriol accurately reflects serum unbound unconjugated estriol and is directly proportional to total serum unconjugated estriol[6] sensitivity.

Saliva Assays in Routine Clinical Practice

Steroid hormones in blood are 95-99% bound to carrier proteins such as SHBG, albumin, and CBG. Saliva levels reflect the unbound fraction, which diffuses freely into tissues, and is often described as the "bioavailable" fraction. In many applications, the bioavailable fraction



Arregger et al. Salivary testosterone: a reliable approach to the diagnosis of male hypogonadism. Clin Endocrinol (Oxf) 2007;67:656-62. © 2007, John Wiley and Sons. Reproduced with permission.

is a more useful clinical parameter than a total serum level. In other applications, such as those where samples must be collected daily or even several times a day, saliva collection is simply more practical. Saliva is preferred to serum in situations where venipuncture stress can affect results, and considered to be the gold standard for collection of adrenal stress hormones in particular. Saliva is also a method of choice for biobehavioral research on hormones and for diurnal cortisol testing, which both require multiple collections making serum testing inconvenient. The simple, non-invasive and flexible collection allowed by ZRT's test kits is ideal for this process.

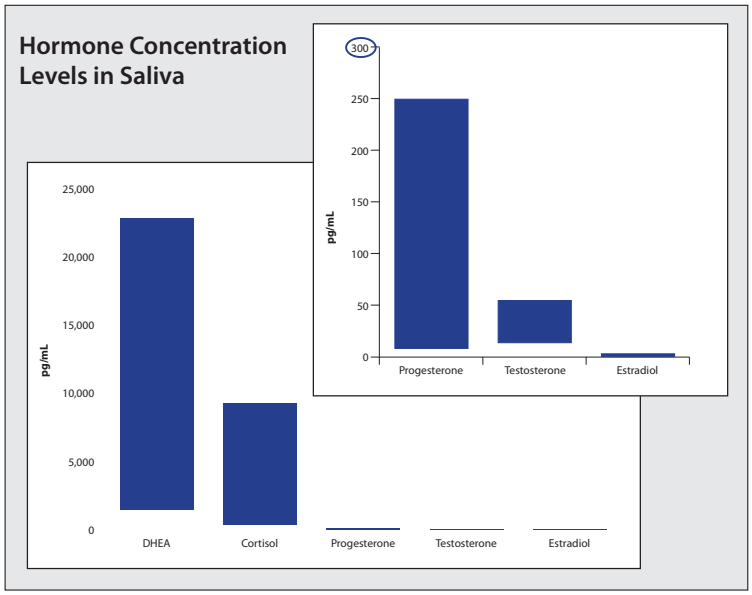
- Salivary cortisol is the method of choice for Cushing's diagnosis[5] and stress assessment[7]
- Saliva estradiol/progesterone is effective for monitoring of menstrual cycles and frequently used in fertility studies[2,3,8,9,10,11,12]
- Salivary testosterone is a reliable method for diagnosing male hypogonadism[1]
- Saliva testosterone is comparable to free plasma testosterone in women with PCOS[13]

The ZRT Difference: Extraction and Accuracy Matters

One of the primary reasons for the misconception regarding the accuracy of saliva testing is specific to estrogen (estradiol, estrone, estriol) testing. This stems from the fact that estrogens are present in very low concentrations in saliva. One study, often referenced in the argument against saliva testing, showed a lack of correlation with estradiol levels measured in saliva as compared with serum. This study used a radioimmunoassay and did not extract the saliva sample to concentrate the estradiol and eliminate potential interfering properties that cause a false positive increase in estradiol levels[14]. The researcher in this case found follicular estradiol values ten times higher than in studies where extraction was used.

Studies that have shown a high correlation between saliva and serum estradiol have employed an extraction step prior to immunoassay[2,3]. However, most laboratories use "direct" assays without extraction, causing accuracy to vary significantly because such assays lack the sensitivity to accurately report low concentrations. ZRT has developed an extraction method followed by an enzyme immunoassay for all 3 estrogens, and results are quantitatively consistent with published studies that show an excellent correlation with serum. Concentrating saliva samples 20-fold prior to analysis in one study[2] gave the excellent serum correlation shown in the graph at far left.

To better understand the need for high assay sensitivity when measuring saliva estrogens, it is helpful to look at levels of estrogens relative to other hormones commonly tested in saliva. The levels of the five hormones regularly tested in saliva are shown in the graph above. The adrenal hormones DHEA and cortisol have the highest concentration, with DHEA commonly ranging from 2,000 to 23,000 pg/mL and cortisol from 400 to 9500 pg/mL. At these levels, the assay is not influenced by background contaminants. However, the sex hormones are present in saliva at much lower concentrations. Progesterone in saliva is only 1/100, while testosterone is roughly 1/1000 and estradiol 1/10,000 the concentration of DHEA.



The increased scale of the graph call-out below clearly demonstrates the relative concentration of progesterone, estradiol, and testosterone in saliva. The ZRT normal reference range for estradiol is only 0.5 to 3.3 pg/mL, compared to DHEA at 2,000 to 23,000 pg/mL. This is the equivalent of comparing the height of the Empire State Building to that of a deck of cards. Viewed from this perspective, it is easy to understand the increased difficulties in accurately measuring estrogens, and the need to test with a lab that has demonstrated the most accurate and reliable testing process available.

At low analyte concentration, background contamination present in saliva has a much greater potential to interfere with assays. This interference is known as the matrix effect. When measuring substances at a relatively high concentration, less sensitive assays give excellent results. But for substances at low concentrations, either a very specific assay must be used, or interfering substances can be removed using extraction methods.

ZRT's saliva tests use an enzyme-immunoassay, but to solve the problem of the matrix effect for hormones at very low concentrations in saliva, we use an extraction

step[15]. This removes the contaminants that interfere with the assay and gives results comparable with those seen in published studies where highly sensitive assays have been used.

Most laboratories don't conduct this extraction step. Some labs don't have the technical knowledge, some do not want to take additional time and effort – or cost – required to conduct this extraction process. However, this process is critical to ensure accurate testing of lower-concentration hormones in saliva.

Ample scientific evidence demonstrates that saliva testing is as accurate as serum. It has the additional advantage of being convenient and non-invasive. Because of the accuracy of saliva testing and the convenience of sample collection, many providers believe saliva testing is the preferred method of hormone testing.

For your introduction to the best in hormone testing, call ZRT Laboratory today at 866-600-1636.

References

1. Arregger AL, Contreras LN, Tumilasci OR, et al. Salivary testosterone: a reliable approach to the diagnosis of male hypogonadism. Clin Endocrinol (Oxf) 2007;67:656-62.
2. Wong YF, Mao K, Panesar NS, et al. Salivary estradiol and progesterone during the normal ovulatory menstrual cycle in Chinese women. Eur J Obstet Gynecol Reprod Biol 1990;34:129-35.
3. Gandia A, Bolufer P, Antonio P, Rodriguez A. Salivary estradiol as a marker of the biological response to induction of ovulation. In: Kirschbaum C, Read GF, Hellhammer DH, eds. Assessment of hormones and drugs in saliva in biobehavioral research, 1992.
4. Meulenberg PM, Hofman JA. Salivary progesterone excellently reflects free and total progesterone in plasma during pregnancy. Clin Chem 1989;35:168-72.
5. Cardoso EM, Arregger AL, Tumilasci OR, Contreras LN. Diagnostic value of salivary cortisol in Cushing's syndrome (CS). Clin Endocrinol (Oxf) 2009;70:516-21.
6. Vining RF, McGinley R, Rice BV. Saliva estriol measurements: an alternative to the assay of serum unconjugated estriol in assessing fetoplacental function. J Clin Endocrinol Metab 1983;56:454-60.
7. Gozansky WS, Lynn JS, Laudenslager ML, Kohrt WM. Salivary cortisol determined by enzyme immunoassay is preferable to serum total cortisol for assessment of dynamic hypothalamic-pituitary-adrenal axis activity. Clin Endocrinol (Oxf) 2005;63:336-41.
8. Belkien LD, Bordt J, Moller P, et al. Estradiol in saliva for monitoring follicular stimulation in an in vitro fertilization program. Fertil Steril 1985;44:322-7.
9. Gann PH, Giovanazzi S, Van Horn L, et al. Saliva as a medium for investigating intra- and interindividual differences in sex hormone levels in premenopausal women. Cancer Epidemiol Biomarkers Prev 2001;10:59-64.
10. Ishikawa M, Sengoku K, Tamate K, et al. The clinical usefulness of salivary progesterone measurement for the evaluation of the corpus luteum function. Gynecol Obstet Invest 2002;53:32-7.
11. Mounib N, Sultan C, Bringer J, et al. Correlations between free plasma estradiol and estrogens determined by bioluminescence in saliva, plasma, and urine during spontaneous and FSH stimulated cycles in women. J Steroid Biochem 1988;31:861-5.
12. Worthman CM, Stallings JF, Hofman LF. Sensitive salivary estradiol assay for monitoring ovarian function. Clin Chem 1990;36:1769-73.
13. Baxendale PM, Jacobs HS, James VH. Salivary testosterone: relationship to unbound plasma testosterone in normal and hyperandrogenic women. Clin Endocrinol (Oxf) 1982;16:595-603.
14. Chatterton RT, Mateo ET, Hou N, et al. Characteristics of salivary profiles of estradiol and progesterone in premenopausal women. J Endocrinol 2005;186:77-84.
15. Newman MS, Stanczyk FZ, Zava DT. Extraction prior to enzyme immunoassay gives reliable salivary estradiol monitoring during estrogen therapy. Society for Gynecologic Investigation 55th Annual Scientific Meeting, San Diego, March 26-29, 2008.