

The need for a better hormone testing model

Physicians have three options for hormone testing – serum, urine, or saliva. Each of these testing methods is a viable option, but has significant limitations, which can be improved upon.

Serum testing

While the most universally accepted testing method (due to the availability of FDA-cleared analyzers that are reliable and inexpensive) serum testing is lacking in some areas. **Adrenal hormones cannot be effectively tested in serum** because you cannot test diurnal free cortisol. There is also **a lack of extensive metabolite testing** for cortisol and estrogens (androgen metabolites are also somewhat limited).

Saliva testing

The primary challenge of salivary testing is the analytical difficulty for testing sex hormones (lab-to-lab comparisons are wildly variable for estradiol, testosterone, and progesterone). Cortisol testing is much easier and most labs can be trusted for accurate analysis. While salivary cortisol values are typically accurate, there is a significant missing piece to surveying a patient’s HA-Axis function – cortisol metabolism. Some conditions (chronic fatigue, for example) leave patients with low free cortisol not because of low production but due to hyper-metabolism. Jerjes (Amer. Psych Soc. 2006) showed that chronic fatigue patients showed cortisol metabolism rates 50% higher in women and 70% higher in men. **You cannot discriminate between low adrenal producers and hyper-metabolizers with saliva testing.** Additionally, **metabolites of estrogens and androgens are not available in saliva.**

Urine Testing

There are two primary drawbacks to urine testing of hormones. First, the collection is cumbersome and as many as **40% of those who collect, do so in error** (Tanaka, 2002). Often times urine that should be part of the 24-hour collection is missed. Additionally, patients often measure the 24-hour volume inaccurately. **Dysfunction in the diurnal pattern of cortisol cannot be ascertained from a 24-hour collection.** The daily free cortisol pattern was formerly only available with salivary cortisol analysis.

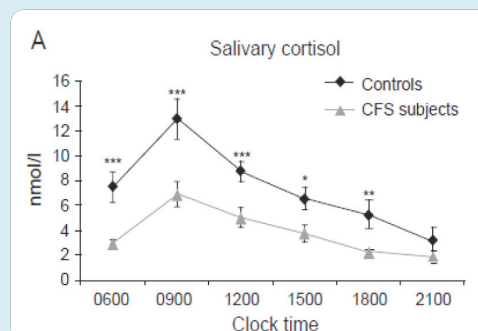
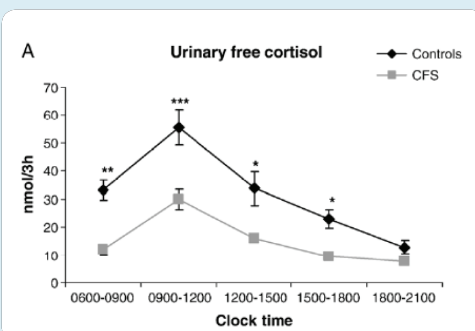
A Better Way

We have developed **a new paradigm in hormone testing.** Collections (4-dried urine samples) are simple and easy, and the diurnal pattern of free cortisol (and cortisone) is combined with an extensive metabolic profile to give **a uniquely comprehensive hormonal overview** with **accurate and precise LC-MS/MS and GC-MS/MS measurements** of all hormones.



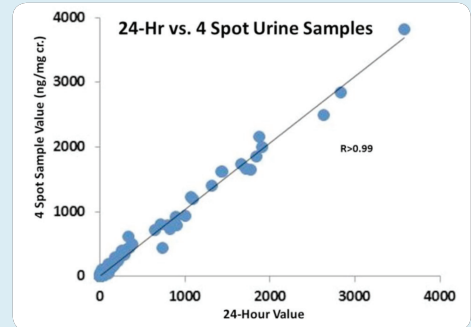
Validating the Concept

Is there literature support for measuring diurnal free cortisol in urine? Jerjes (2005, 2006) studied the diurnal pattern of free cortisol in chronic fatigue patients in both saliva and urine, finding very good agreement (see graphs below). Urine collections have the added value of representing at least two hours of time for each collection. This makes them less influenced by transitory shifts in cortisol levels due to acute minor stressors.



Do values compare favorably to 24-hour collections?

If done properly, the correlation to 24-hour collections is excellent (see graph, right). Because the four dried samples span about 12-14 hours of the day (6-8 hours overnight plus 2 hours per day collection), they represent the entire day's hormone production. A weighted average of the four samples is combined and measured for all hormones other than cortisol and cortisone. Values must be presented relative to creatinine (ng per mg of creatinine) to correct for hydration. This replaces the 24-hour value. The correlation to 24-hour collections is more than reasonable making our model a very respectable alternative to 24-hour collections. With the addition of diurnal free cortisol, it becomes an improvement.

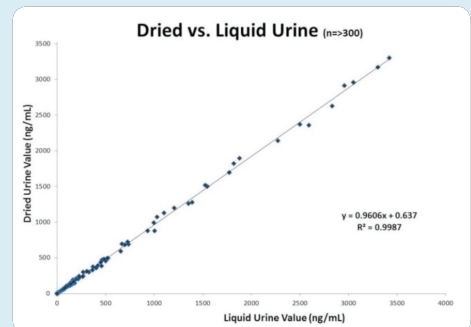


Do dried samples compromise the analysis?

Dried samples work well for hormones and values correlate to liquid samples (see graph, right). Samples are more stable once they are dried and also much easier to ship than liquid samples.

Does the test have limitations?

- Because the test is based off of creatinine measurements, proper kidney function is assumed. The test is not appropriate for those with kidney or creatinine issues.
- Vaginal hormones USUALLY do not work with urine testing because of the local contamination of urine with free hormones placed in the vagina. Our testing is unique. We separate free and conjugated hormones, **so that vaginal hormones can be monitored using our testing without falsely elevated levels due to contamination.**



Methods Used for Testing

Cortisol and cortisone testing is performed by LC-MS/MS. The remaining analytes are all tested by GC-MS/MS, the most accurate method available. For hormones like testosterone, DHT, and estrogen metabolites, GC-MS/MS is has advantages over both GC-MS and LC-MS/MS in terms of accuracy and specificity. Most serum and saliva assays are performed using immunoassays. As a general rule immunoassays are surpassed by mass spectrometry (GC-MS, GC-MS/MS, LC-MS/MS) techniques with respect to accuracy, particularly for low-level compounds like testosterone and estrogens. When patients take exogenous hormones, testing accuracy becomes even more difficult with the use of immunoassays.

All Nordic Laboratories testing methods go through a rigorous validation process to verify accuracy, precision, recovery, linearity, etc. We pride ourselves in relentlessly pursuing the most accurate and precise techniques available for testing



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