A Comparison of Saliva & Wet Urine for Hormone Measurements

Introduction

The evaluation of circulating hormone levels through laboratory testing is an essential part of the assessment and diagnosis of many conditions and diseases. The patient's health and well-being depend on the accuracy and reliability of the laboratory data and health professionals need to be wary of using misleading or inaccurate testing methods.

Hormones can be tested using saliva, blood (serum), and urine (with fingernail and hair rapidly evolving as mediums for cortisol). It is important to look at the advantages and disadvantages of each technique and specimen type. This paper will examine concerns regarding urine testing for hormone analysis. Urine testing has specific applications in clinical diagnostics and medical research; however, there are several significant shortcomings that should be acknowledged. The following information touches on pre-analytical issues, clinical relevance, post-analytical interpretation, and critical diagnostic information that can be missed when urine is used for hormone evaluation.

Pre – Analytical

Collection Requirements

Unlike saliva, which can be easily and discretely collected as part of a normal daily routine at work and at home, urine collection can be difficult, especially for women, and compliance can be an issue. The 24-hour urine collection, for instance, is dependent on the patient’s ability to collect ALL urine in a large plastic jug over the course of 24 hours, day and night. The final total volume of urine collected must also be accurately recorded by the patient. After that, a small sample needs to be poured out of the large collection jug into a smaller container for shipment to the laboratory. The results are only useful if all voided urine is collected and carefully mixed inside of the container and the final volume is calculated correctly by the patient.

Elderly patients often find urine collection to be too cumbersome to manage, which compromises the accuracy of the laboratory results if they are unable to follow collection instructions. Patients are known to miss collections, increase consumption of liquids during the 24 hours, and even use alternative containers to collect (other than the one provided by the laboratory), which may result in contaminants getting into the sample. If collection instructions are not followed carefully and completely, 24-hour urine results will be invalid.

Creatinine Correction

In addition to a burdensome collection method, the urine specimen type is also subject to inconsistent hormone secretion. This can occur when patients have an existing kidney disease in which hormones and their metabolites are not appropriately secreted by the kidneys. Some laboratories attempt to normalize
variations in analyte levels through the measurement of urinary creatinine. Creatinine is a breakdown product of creatine phosphate in muscle which is typically produced at a constant rate and generally reflects the hydration status of the patient.

A Creatinine Correction is an adjustment applied to the measured concentration of an analyte, using the creatinine value, to correct for the hydration status of the patient. However this screening tool is not always precise due to variations in muscle mass and factors such as age, height, weight, and sex (1). Even if the patient has normal renal function and urine production, over-hydration can present a problem with assays that report analyte measurements as a ratio with creatinine. At a certain point, if the sample is too dilute, the relationship between hydration and creatinine concentration does not hold up and an accurate hormone measurement cannot be obtained. Besides the complications that are faced during the pre-analytical collection steps, it is important to identify the gaps in clinical relevance of urinary hormone analysis.

**Clinical Relevance and Reliability**

**Cortisol Testing**

For certain clinical applications of hormone testing, urine laboratory data falls short in providing a complete clinical picture. The salivary free cortisol measurement is a well established technique for evaluation of the hypothalamic-pituitary-adrenal (HPA) axis. Cortisol influences many systems including metabolism, stress adaptation, and immune function. Thorough, time-specific analysis of cortisol is required to capture any deviation from the normal diurnal rhythm of expression. In place of a 24-hour urine collection, measuring free cortisol in saliva is a well known, and a potentially superior method for screening cases of Cushing's syndrome and other hyper- and hypocortisolism states. Saliva has the distinct advantage of being able to capture real-time concentrations with ease.

Urinary Free Cortisol (UFC) is not optimal for an initial screening test for Cushing's syndrome for several key reasons. UFC is often tested from 24-hour urine collection, but because this test relies on detecting increases in daily free cortisol being filtered by the kidneys, specific peaks in cortisol may be missed. Furthermore, morning cortisol levels are not elevated in all patients with Cushing's syndrome, whereas late night cortisol is usually increased (2). Breaking down the cortisol measurement into specific time point collections will help isolate these elevated values instead of masking them within the pooled average of a 24-hour collection.

Additionally, pseudo-Cushing's states can produce false positives with UFC testing. Obesity-related hypercortisolism, as well as pharmaceutical interferences, can produce false positive results (3). At 100% specificity, the sensitivity of UFC for Cushing's syndrome may only be 45-71% whereas salivary cortisol measurement has exhibited 90-95% sensitivity (2,3,4). Salivary cortisol serves as a practical and accurate screening test for Cushing's syndrome. (4,5)

Hypocortisolemia, or low circulating cortisol, is a characteristic of Addison's disease in which the adrenal glands do not produce adequate levels of cortisol. The diagnosis of this disease is not possible from 24-hour urine samples and requires injection of the hormone ACTH to detect stimulation of cortisol production in serum. ACTH stimulation is essentially a “spiking” of the samples to show artificially
elevated levels of cortisol. Saliva has also been tested in multiple studies as a possible alternative to serum and promising correlation has been demonstrated (6).

**Diurnal Rhythm and Cortisol Awakening Response (CAR)**

While incidence of Addison’s disease and Cushing’s syndrome is very low, analysis of cortisol for identifying HPA axis dysregulation to improve the health and well-being of patients is essential. As mentioned, a normal HPA axis is only confirmed through measuring the distinct circadian pattern of cortisol secretion. This pattern encompasses two separate phases that will be discussed below.

The initial Cortisol Awakening Response (CAR) is the rise in cortisol levels observed at 30 minutes post-waking followed by an expected decline one hour after waking. The behavior of cortisol is an important physiological response to anticipation of the day ahead. The ability to measure the waking, 30 minutes post-waking, and 60 minute post-waking cortisol results allows the capture of this dynamic rise and decline which can be combined with testing for the steady downward slope of cortisol throughout the day (7). Measuring the CAR requires a real-time, “snap shot” measurement, which is not feasible with urine due to the accumulation of hormone in the bladder before collection.

Secondly, the cortisol expression follows a distinctive decline throughout the rest of the day. While 24-hour urine collections provide the total free cortisol production over the course of the day, they do not account for peaks and troughs in hormone production nor the diurnal slope that occurs. Diurnal rhythm captured through spot urine collections at certain time points do not provide a snapshot but a less meaningful, averaged urinary hormone output since the previous collection. Saliva collection has the advantage of providing real-time assessment of physiological conditions and responses.

It is believed that these two phases of cortisol production, the CAR and the diurnal rhythm, are interrelated and influence distinct aspects of HPA axis function (8). The ability to map time-specific cortisol values is essential for visualizing the unique pattern of disruption which is then put in context of the overall clinical picture and condition being assessed.

**Hormone Metabolites**

Although measurement of urinary hormone metabolites can have clinically relevant applications, the interpretation of the laboratory data can be difficult and misleading. The output of pre and post metabolized forms of hormones collected can vary greatly between individuals and genders and requires a skilled understanding (9). Reliable results are contingent upon a normal renal clearance rate of these metabolites. As mentioned previously, patients with impaired renal function or a diagnosed kidney disease do not excrete urine or metabolic products normally, so a urine hormone test is not a suitable method in these instances (10).

Furthermore, certain laboratories claim that cortisol metabolite information can be used to understand underlying pathologies associated with atypical cortisol load; however, clinicians should tread carefully when interpreting the results. While it is understood that scientific research has linked certain conditions to the cortisol clearance rate and enzymes needed for metabolism of cortisol, not all urinary hormone tests are well established methods for detecting these conditions or monitoring treatment strategies.
Conditions that affect the clearance of cortisol include extreme malnutrition and early-life stress, hypothyroidism, depression, and insulin resistance (11). The literature available is limited and these data only partially explain anomalies that exist between actual and expected levels (i.e. salivary cortisol values). In order to understand the influence of cortisol clearance rate on an individual's HPA axis function and status, more rigorous data and research is required to generate solid connections and interpretations.

**Additional Advantages of Saliva Testing**

**Dim Light Melatonin Onset (DLMO)**

Circadian rhythm sleep disorders are readily evaluated with a key technique called Dim Light Melatonin Onset (DLMO). For this test, patients stay in a dim light setting (to minimize suppression of melatonin production) and collect samples in 30 minute intervals prior to bedtime. The accuracy of this test depends on the environment and timing of collections. Saliva collection is a simplified and proven method for measurement of melatonin for DLMO patterns (12,13). Urine collections are not a feasible alternative to this application of salivary melatonin analysis. Not only can samples not be collected in the proper environment, but urine production may not be possible in such rapid intervals due to limitations of the patient's ability to void. Furthermore, urine may not reflect the "in-circulation" endogenous levels of melatonin because urine only provides the breakdown product of melatonin, the metabolite 6-sulphatoxymelatonin. Standard bedtime routine of the patient should be followed to produce valid results as well as measurement of the small phase changes, best suited for saliva.

**Female Hormones and Hormone Replacement Therapy**

For estrogens and progesterone in women, saliva testing is an invaluable tool for measuring the dynamics of hormone patterns throughout the menstrual cycle. Moreover, monitoring replacement hormone therapy and checking for imbalances is well established with saliva. Additionally, topical hormone regimens can be misrepresented and inaccurate when monitored with the wrong specimen type.

Serum and urine assays often significantly underestimate the affect of topical hormones such as skin creams and patches. This can result in harmful affects to the patient if overdosing is the result of misinterpretation of the laboratory data (14). A paper by Stanczyk et al. describes how progesterone transdermal creams are absorbed through the skin into the blood stream and the hormones bind to red blood cells due to their affinity to lipids (fat) over aqueous (water) interaction. Literature studying pre and post menopausal women has demonstrated that there is no significant increase in urine excretion of either progesterone or pregnanediol-3-glucuronide after application of progesterone cream. Typically, progesterone is metabolized and conjugated in the liver to form the water-soluble pregnanediol-3-glucuronide, which is excreted in urine; but monitoring topically applied progesterone with urine does not reflect true exposure of other tissues (15). Saliva hormones more accurately reflect tissue uptake and response to replacement hormones delivered through these topical methods (14,16). The salivary response is significant and prompt and indicates that transdermal hormone replacement therapy is most affectively monitored with salivary analysis.
Conclusion

When evaluating laboratory data for hormone analysis, clinicians need to be aware of all of the applications and complications associated with each sample type. In addition to the convenience for the patient, tests should demonstrate a strong clinical relevance within the medical and research communities.

Urinary hormone measurement has revealed concerns that have significant implications on patient satisfaction and treatment. Collection complications and compliance issues, limitations on collection timing, and a lack of consensus on analytical calculations and interpretations are a few notable examples. Many of these downsides can be circumvented if salivary hormone testing is performed in its place.

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Summary Comparison of Common Sample Types:

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Saliva</th>
<th>Wet Urine</th>
<th>Blood</th>
<th>Dried Urine</th>
</tr>
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<tbody>
<tr>
<td>Peer reviewed studies to support testing of hormones</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Assay validation criteria met</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Maybe*</td>
</tr>
<tr>
<td>Clinical applications supported by research</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Stability of analytes demonstrated</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Ability to test free, bioactive hormones</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Measures total hormone load over 24 h</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Convenient collection</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes**</td>
</tr>
<tr>
<td>Measures real-time hormone levels</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

* While the other sample types are well-established globally with common validations, dried urine’s validations remain unavailable given their LDT status.

** Urinating on paper may be convenient, however the quality of the paper and risk of contamination while the sample is drying are of concern, as explained in the text.
Peer reviewed literature to support hormone testing in different sample types

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Saliva</th>
<th>Wet Urine</th>
<th>Blood</th>
<th>Dried Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free diurnal rhythm of cortisol</td>
<td>&gt;1000 citations</td>
<td>2 citations by same group</td>
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<td>0</td>
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<td>Measurement of Cortisol Awakening Response</td>
<td>&gt;450 citations</td>
<td>not feasible</td>
<td>not feasible</td>
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<tr>
<td>Melatonin for DLMO patterns</td>
<td>&gt;75 citations</td>
<td>not feasible</td>
<td>~39 citations</td>
<td>0</td>
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<tr>
<td>Relevant clinical applications of free cortisol</td>
<td>&gt;900 citations</td>
<td>&gt;800 citations</td>
<td>&gt;5600 citations</td>
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<td>Relevant clinical applications of hormone metabolites</td>
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<td>80 citations</td>
<td>&gt;100 citations</td>
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</table>

References


