

ABOUT DRIED URINE TESTING

Urine testing is a common diagnostic method for a wide variety of analytes. ZRT Laboratory uses dried urine for testing elements (iodine, selenium, bromine, arsenic, and mercury) that directly or indirectly impact thyroid hormone synthesis and intracellular conversion of thyroxine (T4) to triiodothyronine (T3). We have also developed dried urine tests for measuring steroid hormone metabolites, giving doctors a tool to look at hormone bioavailability and metabolic pathways that can highlight risk factors for hormone-dependent cancers. In addition, we offer a test for the primary metabolite of melatonin, MT6s, which is included in both the Sleep Balance Profile and the Complete Urine Metabolites profile.

Dried Urine versus Liquid Urine

Collection of urine over a 24-hour period has provided a convenient means to measure the total output of steroid hormones and other analytes during a day. Physiological hormone production fluctuates throughout the day, so hormone testing in a 24-hour urine only provides an average of hormone synthesis, but does not account for peaks and troughs or diurnal variations that occur throughout the day. For example, while total cortisol output can be estimated with a 24-hour urine test, the circadian rhythmic pattern of cortisol production cannot be evaluated with this collection method.

The collection of a 24-hour urine can be messy, and accuracy is dependent on collecting ALL urine in a large plastic jug during the course of the day, accurately measuring the total volume of urine collected, and carefully pouring a small amount of the entire contents of the 24-hour collection into a smaller vial for shipment to the testing laboratory. Urinating directly into a plastic jug is not a simple task for most women, nor is guesstimating the volume collected or pouring a small amount of it from the large plastic jug into a smaller tube. Moreover, collections are often missed, especially those associated with a bowel movement.

ZRT Laboratory has been instrumental in developing a simple, more convenient, and more accurate alternative to liquid urine collection and testing. It involves collecting urine on a filter strip and allowing it to dry. Urine is collected four times throughout the day. The “dried urine” method not only simplifies the collection procedure for patient convenience and compliance but also allows for ease of shipment without concern for sample stability. Urine collection at four time points also overcomes the limitations of interpreting a 24-hour urine collection for hormones that display a well-defined diurnal rhythm (e.g., cortisol and melatonin).

Advantages of Dried Urine Testing Compared to a 24-hour Urine Collection

1. Simple sample collection.

The collection procedure is simple and can be completed at home. A laboratory-grade filter paper strip is saturated with urine either by dipping it into urine that has been collected in a cup, or by urinating directly onto the strip. It takes very little urine to saturate the strip – only about 2 mL – so it isn't necessary to have a full bladder before collecting the sample. After saturating the strip, it is then taped to a towel rack or other object from which it can hang down to dry without the strip itself touching anything.

2. Convenience of shipping.

After at least 4 hours of drying, the sample is ready to be placed in the collection kit box and mailed back to ZRT Laboratory for analysis. No refrigeration is required and shipping costs are low since samples can be sent in the regular mail. This makes shipment to the laboratory more convenient and allows for shipments from anywhere in the world without

Dried Urine Testing.

Non-invasive home test kit.

preservation (frozen or ice packs). Liquid urine is subject to bacterial contamination, and must therefore be shipped refrigerated or frozen.

3. Sample stability.

ZRT Laboratory runs long-term stability experiments under different environmental conditions for each of the analytes we test. Results of these experiments show that urine dried on filter paper can be left at room temperature for at least a month without compromising test results, and samples can be stored frozen indefinitely. This is an advantage over liquid urine samples, where analytical results may be compromised if there are delays in shipment and/or if the samples are not kept refrigerated or frozen.

4. Multiple collections

Urine can easily be collected at specific time points throughout the day allowing for evaluation of the circadian rhythms of hormones such as cortisol and melatonin. The specific time points required are convenient, avoiding the risk of missed collections.

5. Creatinine correction.

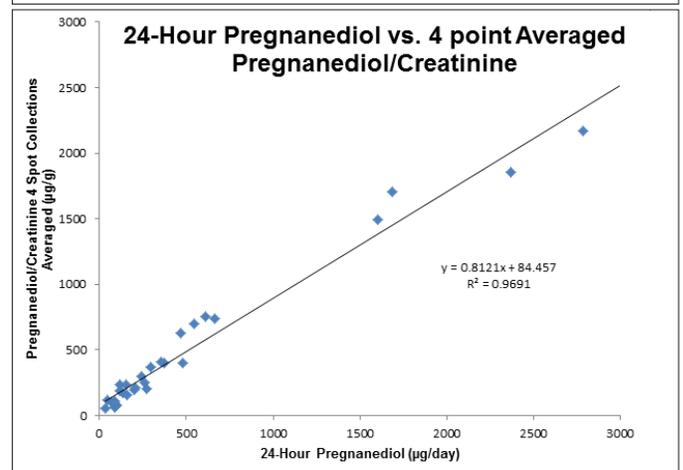
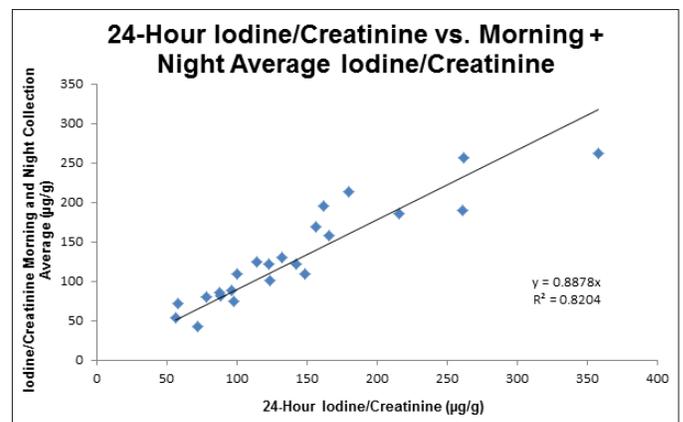
Because urine varies in dilution depending on the time of day or the volume of liquid consumed, all dried urine samples are also tested for creatinine. Creatinine is a natural breakdown product of creatine phosphate in the muscle tissue which is produced at a fairly constant rate. The more dilute the urine, usually as a result of higher liquid intake, the lower the creatinine level. Using creatinine as a correction factor¹ is a very reliable method except in cases of very poor kidney function. All results are therefore expressed in micrograms of the hormone per gram of creatinine, which is more accurate than reporting hormone levels per unit volume, or per 24 hours.

Testing Accuracy

Dried urine has proven to be accurate throughout a wide range of analyte concentrations. Dried urine samples are extracted and analyzed in the laboratory using highly accurate methods including liquid chromatography/tandem mass spectrometry (LC-MS/MS; used for free cortisol, free cortisone, and MT6s),

gas chromatography/tandem mass spectrometry (GC-MS/MS; used for steroid hormone metabolites following enzyme digestion and derivatization to increase sensitivity), inductively-coupled plasma mass spectrometry (ICP-MS; used for elements testing^{2,3}) and a direct mercury analyzer for mercury testing. For elements testing, ZRT Laboratory uses external controls with certified analyte concentrations to ensure accuracy.

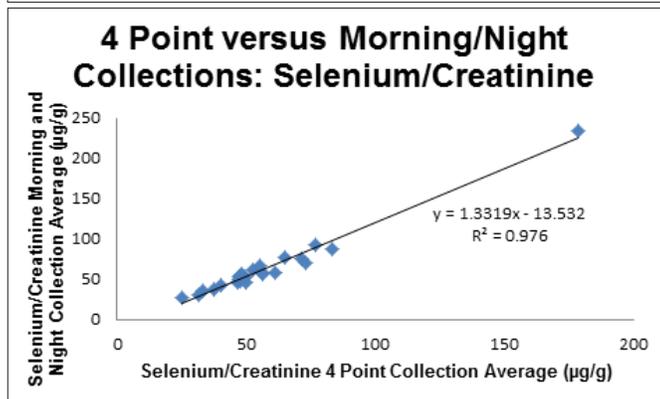
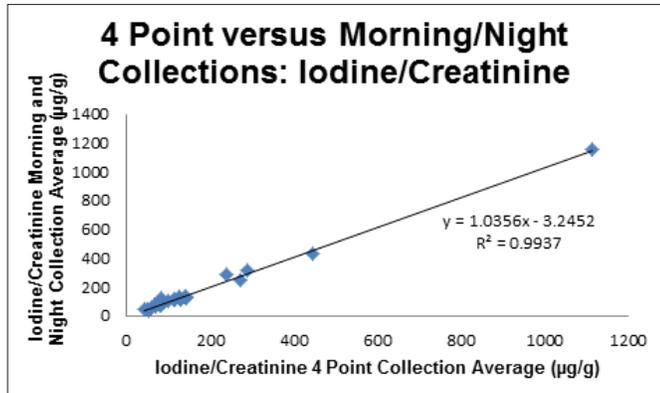
ZRT Laboratory has carried out extensive validation studies showing that the dried urine test shows an excellent correlation with standard 24-hour collections (see graphs below).



Sample Collection for Elements Testing

ZRT's elements testing requires only two dried urine samples: the first morning void after waking and the last urine void before bedtime. Our research at ZRT

Laboratory has shown that these two samples, when combined, give results quantitatively equivalent to four combined spot collections taken throughout the day (see graphs).



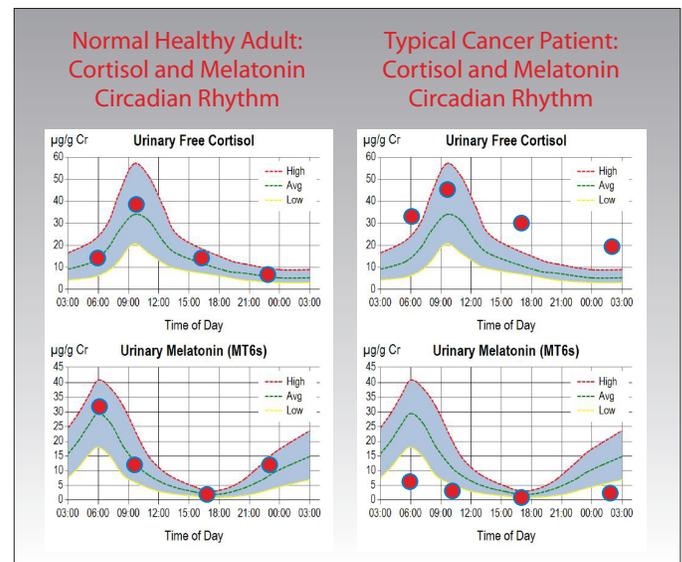
Sample Collection for Metabolites Testing

The steroid hormone metabolites and the melatonin metabolite (MT6s) require four spot collections: first morning void, second morning void (preferably within 2 hours of rising), evening between 5 and 7 p.m. (prior to the evening meal) and last thing before bedtime. Four collections are required because of the diurnal rhythm of hormone secretion, particularly important for the urinary free cortisol and the MT6s tests, and these time points best portray the expected diurnal patterns of cortisol and melatonin. The four collections are also combined to provide an average of the hormone levels seen in a 24-hour collection. These collections not only capture the peaks and troughs of cortisol and melatonin levels seen throughout the day, but also allow for convenience of collection (first awakening, just before leaving for work, returning from work before dinner, just before bed at night).

Unique Advantages of Dried Urine for Cortisol and Melatonin Testing

ZRT has established expected cortisol and melatonin ranges and the four point test results are plotted as seen in the graphs below. The graphs on the left show what is expected for the diurnal levels of urinary cortisol and melatonin (MT6s) in a healthy individual where urinary cortisol is low in the morning, rises several hours later, and then gradually falls to a low point before bed at night. In contrast to urinary free cortisol (UFC), the urinary melatonin metabolite MT6s peaks in the early morning void which reflects the night resting phase. Melatonin then drops throughout the day, reaching a low point in the evening (reflects the greatest exposure to light) and then begins to rise with reduced lighting.

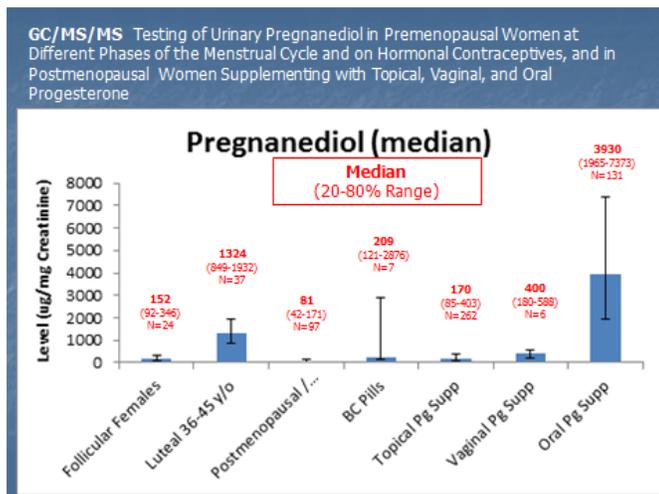
The graphs on the right are typical of these patterns in a cancer patient where the diurnal cortisol patterns



are flat and very little melatonin is produced at night. A distinct advantage of the diurnal urine melatonin versus salivary diurnal melatonin is that in the urine collection the first void is the sum of all melatonin produced during the “dark” phase, when melatonin production should be at its highest. To evaluate salivary melatonin at peak of production requires waking at 2-3 a.m. to collect saliva, which is extremely inconvenient and results in poor compliance.

Development of Ranges for Steroid Metabolites

ZRT has established specific hormone ranges for gender, menopausal status (premenopausal follicular and luteal; postmenopausal), and exogenous hormone supplementation as it relates to hormone type, dosage, timing, and delivery (e.g., oral, topical, sublingual, patch, etc.). This is particularly important as different methods of exogenous hormone delivery can result in remarkably different levels of the hormone in urine. An example of the variation in the ranges of pregnanediol, as it relates to these parameters, is seen in the diagram below. As seen in this graph, topical progesterone delivery raises urinary pregnanediol very little, while oral progesterone raises pregnanediol levels beyond luteal phase levels.



References

1. Barr DB, Wilder LC, Caudill SP, Gonzalez AJ, Needham LL, Pirkle JL. Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. *Environ Health Perspect.* 2005;113:192-200.
2. Zava TT, Kapur S, Zava DT. Iodine and creatinine testing in urine dried on filter paper. *Anal Chim Acta* 2013;764:64-9.
3. Zava TT, Zava DT. Determination of iodine, bromine, selenium and arsenic by ICP-DRC-MS using urine dried on filter paper. Poster presented at the 83rd Annual Meeting of the American Thyroid Association, October 16-20, 2013, San Juan, Puerto Rico.