

# DRIED URINE TEST SPECIFICATIONS

## Free Cortisone

### Clinical Information

Cortisone is the inactive form of cortisol. Cortisol is reversibly metabolized to cortisone in tissues by the actions of 11 $\beta$ -hydroxysteroid dehydrogenase types 1 and 2. The relative amounts of cortisol and cortisone are therefore determined by the activities of these enzymes, which can be affected by compounds such as licorice and carbenoxolone. Measurement of both hormones gives a better picture of total cortisol production by the adrenals.

Cortisol and cortisone levels reflect hypothalamic-pituitary-adrenal (HPA) axis function and show a diurnal variation linked to the sleep/wake cycle, so that levels are normally at their lowest during the night, rising to a peak about 30-60 minutes after waking, and then falling again gradually throughout the day. Four-point diurnal free cortisone, graphed on test reports, indicates the pattern of cortisone production throughout the day. Free cortisone in urine represents the bioavailable fraction that is not protein bound in the circulation.

Urinary free cortisone levels are corrected using urinary creatinine to allow for variations in hydration status. Reference ranges were established at 4 diurnal time points: 1st morning void, 2nd morning void (2 h after the 1st, capturing the early morning peak production), evening (just before dinner), and bedtime. At these time points, free cortisone ranges for women and men are: 31.6–91.6, 63.3–175.8, 30.6–88.5, and 15.5–44.7  $\mu\text{g/g}$  creatinine, respectively.

### References:

Jerjes WK, Peters TJ, Taylor NF, et al. Diurnal excretion of urinary cortisol, cortisone, and cortisol metabolites in chronic fatigue syndrome. *J Psychosom Res.* 2006;60:145-53.  
Basta M, Chrousos GP, Vela-Bueno A, Vgontzas AN. Chronic Insomnia and the Stress System. *Sleep Medicine Clinics* 2007;2:279-91.  
Buckley TM, Schatzberg AF. On the interactions of the hypothalamic-pituitary-adrenal (HPA) axis and sleep: normal HPA axis activity and circadian rhythm, exemplary sleep disorders. *J Clin Endocrinol Metab.* 2005;90:3106-14.

### Assay Method: LC-MS/MS

#### Intra-assay Precision

Intra-assay precision was determined by analyzing method controls spanning the reference range for cortisone 8 times within the same run. Results are shown below:

Mean Cortisone Concentration (ng/mL)	Standard Deviation	Coefficient of Variation (C.V. %)
36.2	5.2	14.3
59.4	2.7	4.6
116.0	12.5	10.8

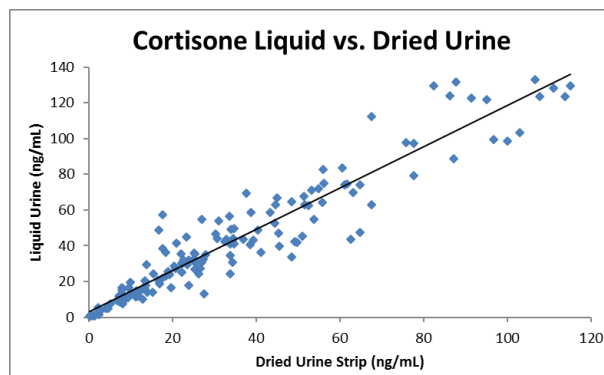
#### Inter-assay Precision

Inter-assay precision was determined by analyzing a group of method controls in 9 different runs. Results are shown below:

Mean Cortisone Concentration (ng/mL)	Standard Deviation	Coefficient of Variation (C.V. %)
10.2	1.2	11.8
34.5	4.7	13.5
109.2	14.4	13.2

#### Accuracy

To test the accuracy of the dried urine assay for cortisone, dried urine samples collected at the same time as corresponding liquid urine samples were analyzed. Resulting correlation data are shown below ( $R^2 = 0.9$ ):



#### Linearity

The free cortisone assay gives excellent linearity over the reportable range of 1.4-350 ng/mL.

#### Analyte Stability

The dried urine free cortisone samples are stable for 30 days at room temperature.

#### Specimen Collection

Kits for dried urine collection contain four filter paper collection strips, easy-to-follow instructions, and a mailer to return the sample for analysis.