

DRIED URINE TEST SPECIFICATIONS

Free Cortisol

Clinical Information

Cortisol is a hormone produced by the adrenal glands in response to physical or emotional stress. It is important for blood sugar control, blood pressure regulation, and immune function. Its 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. Free cortisol in urine represents the bioavailable fraction that is not protein bound in the circulation.

Four-point diurnal free cortisol, graphed on test reports, indicates the pattern of cortisol production throughout the day and shows the effects on adrenal function of abnormal exposure to stress. High cortisol levels throughout the day show HPA axis hyperactivation, while loss of the morning peak indicates adrenal suppression. Diurnal patterns are affected by chronic stress, sleep disorders, PTSD, Cushing's disease, and chronic fatigue syndrome.

Urinary free cortisol 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 cortisol ranges for women and men are: 7.8–29.5, 23.4–68.9, 6.0–19.2, and 2.6–8.4 $\mu\text{g/g}$ 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 cortisol 8 times within the same run. Results are shown below:

Mean Cortisol Concentration (ng/mL)	Standard Deviation	Coefficient of Variation (C.V. %)
19.1	1.9	9.9
22.4	1.5	6.5
62.6	6.4	10.2

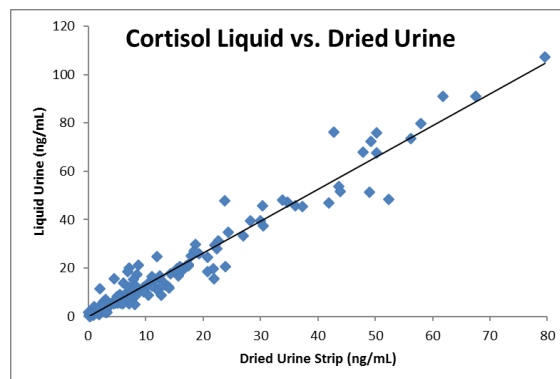
Inter-assay Precision

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

Mean Cortisol Concentration (ng/mL)	Standard Deviation	Coefficient of Variation (C.V. %)
6.1	1.0	16.5
21.2	2.1	9.9
52.6	6.4	12.2

Accuracy

To test the accuracy of the dried urine assay for cortisol, dried urine samples collected at the same time as corresponding liquid urine samples were analyzed. Resulting correlation data are shown below ($R^2 = 0.94$):



Linearity

The free cortisol assay gives excellent linearity over the reportable range of 0.7-275 ng/mL.

Analyte Stability

The dried urine free cortisol 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.