

Cortisol (urine)

1 Name and description of analyte

1.1 Name of analyte

Cortisol

1.2 Alternative names

Hydrocortisone, (11 β)-11,17,21-trihydroxypregn-4-ene-3,20-dione. Previously widely referred to as 'urinary free cortisol' but the use of 'free' may better be reserved for measurements made by immunoassay, and omitted in relation to MS-based methods).

1.3 NLMC code

To follow

1.3 Description of analyte

Cortisol is the major glucocorticoid synthesised from cholesterol in the adrenal cortex. It also has some mineralocorticoid activity, but this is probably only important in pathological states. Synthesis is stimulated by pituitary adrenocorticotrophic hormone (ACTH); ACTH release is stimulated by corticotrophin-releasing hormone (CRH) from the hypothalamus and is inhibited by cortisol (negative feedback). In the circulation, approximately 75% is protein-bound, principally to transcortin (cortisol-binding globulin, CBG). Only unbound cortisol is excreted in urine.

1.4 Function of analyte

During the fasting state, cortisol increases hepatic gluconeogenesis and the peripheral release of substrates, primarily from muscle, required for gluconeogenesis. Cortisol also increases glycerol and free fatty acid release by lipolysis and increases muscle lactate release. Glycogen synthesis and storage by is enhanced by cortisol. Uptake of glucose in muscle and adipose tissue is inhibited by cortisol.

Cortisol also has anti-inflammatory actions through decreasing the migration of inflammatory cells to the sites of injury and inhibiting lymphocyte production.

Cortisol secretion is closely regulated by ACTH, which is secreted in an episodic manner superimposed on a circadian rhythm. Cortisol secretion occurs in parallel to the secretion of ACTH. Cortisol secretion is low in the evening and continues to decline into the first few hours of sleep, after which there is an increase. After waking, an individual's cortisol secretion gradually declines throughout the day, with fewer secretory episodes of smaller magnitude. ACTH (and thus cortisol) secretion is stimulated by stress.

2 Sample requirements and precautions

2.1 Medium in which measured

1. Urine

2. Cortisol can also be measured in serum or heparinised plasma and saliva ([see separate entry](#)).

- 2.2 Precautions re sampling, handling etc.
A 24 h urine collection should be used to determine urine cortisol. Urine can be collected into containers with or without boric acid preservative. Samples collected with preservative should be refrigerated during collection and an aliquot frozen on receipt. The use of a timed overnight collection may be an acceptable alternative.

In Scotland, an overnight urinary cortisol:creatinine ratio is typically used in the initial evaluation of patients with suspected cortisol excess, usually on three occasions.

3 Summary of clinical uses and limitations of measurements

3.1 Uses

Measurement of urine cortisol is used in the diagnosis of Cushing's syndrome, a disorder of hypercortisolism.

3.2 Limitations

Repeated measurement of urine cortisol may be required as some patients with Cushing's syndrome may have non diagnostic urine cortisol values. Normal urine cortisol excretion on three separate occasions excludes Cushing's syndrome. Repeated measurements may be necessary in the early stages when there may be episodic hypersecretion and also in cases of cyclical Cushing's, where there are periods of spontaneous remission (see 6.2).

4 Analytical considerations

4.1 Analytical methods

a. Immunoassay: cortisol is extracted from the urine, typically using an organic solvent. After drying down, the sample is re-constituted and analysed by immunoassay. Immunoassay methods are subject to variable interference from other steroids and their conjugates; the EQA data is poor.

b. Chromatographic methods: HPLC, and more frequently LCMS, are increasingly used to measure urine cortisol. These methods are more specific for cortisol than immunoassays.

4.2 Reference method

Isotope dilution mass spectrometry

4.3 Reference material

Cortisol (hydrocortisone) (Standard Reference Material (SRM) 921) available from the National Bureau of Standards, Washington DC, USA.

4.4 Interfering substances

Cross-reactivity occurs when measuring urine cortisol by immunoassay owing to other naturally occurring steroids and their conjugates as well as some synthetic glucocorticoids e.g. , prednisolone and prednisone and their metabolites.

4.5 Sources of error

1. Solvent urine extraction is technically demanding and does not remove all interfering steroids and conjugates; immunoassay methods show wide

imprecision.

2. Incomplete 24 h urine collection is a major potential source of error in the determination of urine free cortisol. Measurement of urine creatinine may help to assess the completeness of collection.

3. Studies have shown wide variation in results produced by different methods.

5 Reference intervals and variance

5.1.1 Reference interval (adults)

Urine cortisol (by immunoassay): 55–248 nmol/24 h

The reference ranges for LCMS methods are generally 50% of immunoassay – see Appendix for published LCMS reference ranges.

5.1.2 Reference intervals (others)

Urine cortisol:

1–10 years 6–74 nmol/24h

11–20 years 14–152 nmol/24h

Urine cortisol:creatinine ratio:

Adults: <25 µmol/mol

Children <10 yrs: <40 µmol/mol

5.1.3 Extent of variation

5.1.3.1 Interindividual CV: no data identified

5.1.3.2 Intraindividual CV: no data identified

5.1.3.3 Index of individuality: not available

5.1.3.4 CV of method: ranges between no data available

5.1.3.5 Critical difference: no data available

5.1.4 Sources of variation

Urine cortisol excretion is influenced by changes in binding protein concentration. For example, in pregnancy, CBG concentrations increase and so there is increased capacity to bind cortisol, thus decreasing the amount of cortisol available for excretion. The hydration status of the subject and renal disease also influence urine cortisol excretion. Such factors affect the relationship between cortisol production and its urinary excretion.

6 Clinical uses of measurement and interpretation of results

6.1 Uses and interpretation

1. Measurement of urine cortisol in a 24 h urine sample is a useful screening test for hypercortisolaemia. In states of cortisol excess, the binding capacity of CBG is exceeded thus increasing the plasma free cortisol and consequently urine excretion of cortisol. Measurement of urine cortisol has no use in the diagnosis of hypocortisolism as the methods lack sensitivity at low cortisol concentrations. Furthermore, a low urine cortisol may be found in healthy individuals.

6.2. Confounding factors

1. Cortisol production and therefore urinary excretion increases during stress, for example during surgery, acute illness and following trauma. Production (and hence excretion) may be reduced in starvation and in pregnancy.

2. Incomplete 24 h urine collections may affect the validity of the results.

3. Cross reactivity in immunoassay can lead to falsely elevated results.

4. Repeated measurement of urine cortisol may be required as some patients with Cushing's syndrome may have non-diagnostic urine free cortisol values. Normal urine cortisol excretion on three separate occasions excludes Cushing's syndrome.

7 Causes of abnormal results

7.1 High concentrations

7.1.1 Causes

High concentrations are typical of Cushing's syndrome (corticosteroid excess) but can also occur in severe depression and alcoholism and when cortisol or ACTH are given therapeutically

7.1.2 Investigation

Suspected Cushing's syndrome should be investigated in two stages.

1. Screening tests should be employed to document the presence of hypercortisolism:
 - urine cortisol excretion
 - low dose dexamethasone suppression test ([see separate entry for cortisol](#)).
2. Diagnostic tests are used to determine the cause of cortisol overproduction. These include:
 - plasma ACTH measurement: low [ACTH] suggests an adrenal cause, whereas normal/ high [ACTH] suggests ectopic ACTH secretion or pituitary hypersecretion of ACTH (Cushing's disease)
 - high dose dexamethasone suppression test (dexamethasone 2 mg 6-hourly for 48 h followed by measurement of plasma cortisol: failure to suppress cortisol secretion suggests ectopic ACTH secretion or an adrenal cause; in Cushing's disease, [cortisol] typically decreases to <50% of the pre-treatment value
 - corticotrophin releasing hormone (CRH) test: (CRH 100 µg i.v.with measurement of cortisol after 60 minutes): in Cushing's disease, there is typically an increase in plasma [ACTH] and [cortisol]; in ectopic ACTH secretion or adrenal tumours, there is typically no response
 - selective venous sampling; [ACTH] is measured in inferior petrosal vein samples before and after CRH stimulation. This test is technically demanding but is valuable in diagnosing pituitary-dependent Cushings. It can be used to lateralise the pituitary source in ~70% cases. Suppressed [ACTH] in both petrosal samples suggests a non-pituitary source of ACTH.
 - imaging: CT scanning of the adrenal glands and MRI of the pituitary gland can help identify tumours.

7.2 Low concentrations

7.2.1 Causes

Measurement of urine cortisol is not indicated in the investigation of disorders of hypocortisolism owing to lack of sensitivity at low concentrations. Low urine cortisol excretion may be found in healthy individuals.

7.2.2 Investigation

None required

- 7.3 Notes
Exogenous administration of corticosteroids, which can cause the clinical features of Cushing's syndrome but suppress normal cortisol secretion, should always be eliminated before instituting tests for adrenal dysfunction.

8 Performance

- 8.1 Sensitivity, specificity etc. for individual conditions
The sensitivity of urine cortisol for the diagnosis of hypercortisolism ranges between 76 and 100% and the specificity 95–98%. The variation depends on the reference range and assay employed. (For meta-analysis of value of tests for Cushing's, see 9.1).

9 Systematic reviews and guidelines

9.1 Systematic reviews

1. Accuracy of diagnostic tests for Cushing's syndrome: a systematic review and meta-analyses. Elamin MB, Murad MH, Mullan R *et al.* J Clin Endocrinol Metab. 2008;93:1553-1562.

Evidence on the accuracy of common tests for diagnosing Cushing's syndrome is summarised. The review found that commonly used tests to diagnose Cushing's syndrome appear to be highly accurate in referral practices with samples enriched with patients with Cushing's syndrome. Their performance in usual clinical practice remains unclear

9.2 Guidelines

1. The diagnosis of Cushing's syndrome: an Endocrine Society Clinical Practice Guideline. Nieman LK, Biller BM, Findling JW *et al.* J Clin Endocrinol Metab 2008; 93:1526-1540.

Updated recommendations for the diagnosis of Cushing's syndrome.

2. The diagnosis of Cushing's syndrome: an Endocrine Society Clinical Practice Guideline: commentary from a European perspective. Guignat L, Bertherat J. Europ J Endocrinol 2010;163: 9-13

Evidence-based clinical practice guideline for the diagnosis of Cushing's syndrome produced by the American Endocrine Society and European Society of Endocrinology Both articles support the use of urine cortisol measurement as an initial test in the diagnosis of hypercortisolism.

9.3 Recommendations

1. Diagnosis/differential diagnosis of Cushing's syndrome: a review of best practice. Newell-Price J. Best Pract Res Clin Endocrinol Metab 2009;23(S1): S5-14

Review of current practice guidelines taking into account the advantages and pitfalls of the diagnostic tests used in the diagnosis of Cushing's syndrome.

2. Overnight urinary free cortisol determination: A screening test for the diagnosis of Cushing's syndrome. Corcuff JB, Tabarin A, Rashedi M *et al.*, Clin Endocrinol (Oxf) 1998;48:503-508.

Evaluation of overnight urine cortisol:creatinine ratio in the screening of patients with suspected Cushing's syndrome. Sensitivity and specificity were 100% and 97% respectively.

10. Links

10.1 Related analytes
None

10.2 Related tests

Other tests useful in the investigation of adrenal function include [ACTH], principally to determine the cause. High [glucose] and low [potassium] may occur in adrenal hyperfunction (particularly when due to ectopic secretion of ACTH).

Appendix

**Published LC-MS/MS reference range data for urine free cortisol (nmol/24 h)
(data kindly supplied by Dr Helen Field)**

Author and year	Method	Child 1-4 y	Child 3-8 y	Child 9-12 y	Child 13-17 y	Adult females	Adult males	(n)
Leeds in house	Extraction CPB	NA	NA	NA	NA	<305	<305	-
Leeds in house	LC-MSMS	NA	NA	NA	NA	<147 ^a	<147	142
McCann 2005	LC-MSMS	NA	NA	NA	NA	<165 (97.5 th centile); <146 (95 th centile)	<165 (97.5 th centile); <146 (95 th centile)	110
Mayo Clinic, accessed 2012 (Taylor 2002)	LC-MSMS	NA	4-55	7-102	11-155	10-124	10-124	-
Arup, accessed 2012	LC-MSMS	NA	<50	<102	<155	<124	<166	-
Quest, accessed 2012	LC-MSMS	3-23	3-83 (5-9 y)	3-124 (10-13 y)	8-152 (14-17 y)	11-138	11-138	-
Jung JCEM 2011	LC-MSMS	NA	NA	NA	NA	<171 ^b trimester 1, 146-224; trimester 2, 71-303; trimester 3, 108-376; post partum, <155; OCP, 10-218	NA	15 20 12

Key: a = limit of quantitation (10 nmol/L); b = mean \pm 2SD; OCP = oral contraceptive pill; - = no data.

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Date Completed: 1.2013

Date Revised: