Cortisol (serum, plasma)

1 Name and description of analyte

1.1 Name of analyte
Cortisol

1.2 Alternative names
Hydrocortisone, 11β; 17, 21-trihydroxypregn-4-ene-3,20-dione

1.3 NMLC code

1.4 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).

1.5 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 imflammatory 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. Cortisol can be measured in serum or heparinised plasma; cortisol can also be measured in urine (see separate entry).
2. Measurements of cortisol in saliva are used as a surrogate for measurements in serum/plasma.

2.2 Precautions re sampling, handling etc.

1. It is usually recommended that stress should be minimised during venepuncture for cortisol measurement although the importance of doing this has probably been exaggerated.
2. It is recommended that specimens of saliva should be frozen to precipitate salivary glycoproteins and leave a non-viscous liquid.
3. Contamination of saliva with blood invalidates salivary measurements.

3 Summary of clinical uses and limitations of measurements

3.1 Uses

Measurement of cortisol is used primarily to diagnose and monitor the treatment of Addison's disease and to diagnose Cushings syndrome, disorders of hypocortisolism and hypercortisolism, respectively.

3.2 Limitations

The diagnostic utility of a single cortisol measurement is limited by the episodic nature of cortisol secretion, its diurnal variation in concentration and its elevation during stress. Stress may result in patients with adrenal insufficiency having a plasma [cortisol] within the reference range; patients with early Cushing's syndrome may have normal values of [cortisol] during the day despite loss of the diurnal variation. More information is obtained by dynamic testing of the hypothalamic-pituitary-adrenal (HPA) axis.

4 Analytical considerations

4.1 Analytical methods

1. Total [cortisol] in serum/plasma

a. Chromatographic

GC, LC, HPLC and GCMS and LCMS have been used to measure cortisol. These methods have the advantage of specificity in that they distinguish cortisol from other steroids and metabolites. However, the methods are labour intensive and require sample processing before analysis.

b. Immunoassay

This is the most frequently used technique. Cortisol is quantitatively displaced from its binding proteins and measured immunometrically using antibodies supposedly specific to cortisol. In practice, some cross-reactivity, e.g. with 11-deoxycortisol or prednisolone is inevitable.

2. Free [cortisol] in saliva

Cortisol can be measured in saliva by immunoassay or LCMS. Samples do not require extraction prior to analysis, as the salvia contains very little cortisol binding proteins or cortisol metabolites.

4.2 Reference method

Isotope-dilution GCMS. Cortisol is extracted from serum and derivatised. Deuterated cortisol is used as an internal standard.

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 with some synthetic glucocorticoids i.e. prednisolone, methylprednisolone and prednisone.

4.5 Sources of error
Studies have shown significant variation in results produced by different methods.

5 Reference intervals and variance

5.1.1 Reference intervals (adults)
Serum [cortisol]: 09.00 h, 171-536 nmol/L (Roche Elecsys); 00.00h <50 nmol/L.
Salivary [cortisol]: 08.00 h, 4–28 nmol/L; 23.00h <5 nmol/L
5.1.2 Reference intervals (others)
Serum [cortisol]: neonatal reference intervals are dependent on gestational age and time since delivery; 1–16 years (08.00 h) 200–700; (00.00h) <150 nmol/L
5.1.3 Extent of variation
5.1.3.1 Interindividual CV: 45.6%
5.1.3.2 Intraindividual CV: 20.9%
5.1.3.3 Index of individuality: 0.46
5.1.3.4 CV of method typically <3% (serum)
5.1.3.5 Critical difference (serum): 58 nmol/L
5.1.4 Sources of variation
Stress, diurnal variation (see 1.4 and 3.2)

6 Clinical uses of measurement and interpretation of results

6.1 Uses and interpretation
1. Cortisol can be measured during stimulation of the adrenals and/or pituitary in the investigation of adrenal hypofunction. A [cortisol] >550nmol/L makes primary adrenal hypofunction very unlikely. However, when [CBG] are elevated, higher values are required to exclude adrenal insufficiency (see 6.2.3).
2. A midnight serum [cortisol] <50 nmol/L excludes, and a value >200 nmol/L has high diagnostic specificity for, adrenal hyperfunction. Cortisol can also be measured after suppression of ACTH release from the pituitary in the investigation of suspected adrenal hyperfunction.
3. Salivary cortisol is in equilibrium with free cortisol and can be used as an index of free cortisol. It is commonly used in children as an alternative to cortisol measurements in serum. Measurement of a late-night salivary cortisol is becoming increasingly frequently used as an alternative to serum, to determine whether diurnal variation is present in suspected adrenal hyperfunction.

6.2. Confounding factors
1. Cortisol secretion exhibits a circadian rhythm with highest concentrations occurring in the morning and the lowest at around midnight.
2. Cortisol concentrations increase during stress, for example during surgery, acute illness and following trauma.
3. In hyperoestrogenic states, for example during pregnancy, with exogenous oestrogens or with the use of oral contraceptives, [CBG] is

increased resulting in an elevated total [cortisol] to maintain the equilibrium between free and bound cortisol; CBG may also be increased in hyperthyroidism, diabetes and in certain haematological disorders. CBG may be decreased in familial CBG deficiency, hypothyroidism and protein deficiency states such as severe liver disease and nephrotic syndrome.CBG also decreases on recumbancy.

7 Causes and investigation 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.

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:
   - 24 h urine cortisol excretion: this is increased in Cushing's syndrome
   - low dose dexamethasone suppression test (dexamethasone 0.5 mg 6-hourly for 48 h followed by measurement of cortisol: there is a failure of suppression of cortisol secretion in Cushing's syndrome ([cortisol] >50nmol/L). (The overnight suppression test in which cortisol is measured at 09.00 h after dexamethasone 1 mg the previous night is frequently used but is less specific.)
   - Late-night salivary [cortisol]: the diurnal variation in secretion is lost in Cushing's syndrome and nocturnal salivary [cortisol] is raised.
2. Diagnostic tests are used to determine the cause of cortisol overproduction.
   - 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 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 [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. Similar [ACTH] in both petrosal and peripheral vein 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

These are found in adrenal hypofunction, whether of adrenal or hypothalamic/pituitary origin. However, the most frequent cause of a low

[cortisol] is suppression of the pituitary-adrenal axis by synthetic glucocortiocoids given therapeutically

7.2.2 Investigation

1. Basal [cortisol] measurement is of limited value but a value of <50 nmol/L at 09.00 h is effectively diagnostic of adrenal insufficiency, provided the subject is not being treated with synthetic corticosteroids.
2. ACTH stimulation test (250 μg i.m.or i.v. tetracosactrin with measurement of cortisol at 30 minutes): an increase in [cortisol] above 550nmol/L, with an increment >200nmol/L from baseline indicates normal adrenal function. (Alternatively, a single dose of 1mg tetracosactrin is given and [cortisol] is measured after 6h and 24h. A gradual rise in cortisol occurs in normal subjects.)
3. Basal ACTH measurement: a high [ACTH] is indicative of primary adrenal hypofunction, whereas low or normal [ACTH] suggests secondary adrenal hypofunction.
4. Depot ACTH stimulation test: in primary adrenal insufficiency, the adrenals fail to respond to several days of repeated ACTH stimulation (1 mg of depot tetracosactrin daily for 3 days). In secondary and tertiary adrenal hypofunction, the adrenal glands may fail to respond in the short test but an increase in [cortisol] is seen after repeated administration of ACTH.

7.3 Notes

1. The diagnostic utility of a single cortisol measurement is limited by the diurnal nature of cortisol secretion and its elevation during stress. More information is obtained by dynamic testing of the HPA axis.
2. 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

1. Addison's disease is diagnosed on the basis of [cortisol] after stimulation of the hypothalamo-pituitary-adrenal axis; therefore sensitivity and specificity are 100%.
2. The diagnosis of the cause of Cushing's syndrome may require the use of several different tests to determine the integrity of the HPA axis. False positive and false negative results can occur with all diagnostic laboratory tests for adrenal hyperfunction. In some cases, diagnosis can only be established on the basis of selective venous sampling and/or imaging.

9 Systematic reviews and guidelines

9.1 Systematic reviews

1. Meewisse ML, Reitsma JB, de Vries GJ *et al.* Cortisol and post-traumatic stress disorder in adults: Systematic review and meta-analysis. Br J Psychiatry 2007;191: 387-392.
Post-traumatic stress disorder has been associated with lower levels of cortisol in certain conditions. Further work is required to determine if this is related to gender, abuse or the method of measurement.

2. The effect of shift rotation on employee cortisol profile, sleep quality, fatigue and attention level: a systematic review. Niu SF, Chung MH, Chen CH Iet al. J Nurs Res 2011;19:68-81.
There is a reversal in cortisol circadian rhythm after five continuous night shifts leading to poor sleep quality.

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.

9.3 Recommendations

1. The diagnosis and investigation of adrenal insufficiency in adults. Wallace I, Cunningham S, Lindsay J. Ann Clin Biochem 2009;46:351-367.
The role of the laboratory and assay methodology is discussed in the differential diagnosis of adrenal insufficiency.
2. Recommendations for the diagnosis and management of corticosteroid insufficiency in critically ill adult patients: consensus statements from an international task force by the American College of Critical Care Medicine. Marik PE, Pastores SM, Annane D *et al*. Crit Care Med 2008;36:1937-1949
Evidence linked recommendations for the diagnosis and management of adrenal insufficiency in critically ill patients.

10. Links

10.1 Related analytes

Measurement of 17-hydroyprogesterone is used in the diagnosis of the commonest form of congenital adrenal hyperplalsia, a condition that usually presents with adrenal failure in early infancy but can present at any age. In adult females, it presents more frequently with features of e xcess androgen excretion than of cortisol deficiency.

10.2 Related tests

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

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