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

1.4 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 adrenocorticotropic 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 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. 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 Cushing's 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 <5nmol/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: 20.9%
5.1.3.2 Intraindividual CV: 45.6%
5.1.3.3 Index of individuality: 2.18
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 glucocorticoids 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.)


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 hyofunction, 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


*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.*

There is a reversal in cortisol circadian rhythm after five continuous night shifts leading to poor sleep quality.

9.2 Guidelines

*Updated recommendations for the diagnosis of Cushing’s syndrome.*


*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.

*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.

*Evidence linked recommendations for the diagnosis and management of adrenal insufficiency in critically ill patients.*

10. Links

10.1 Related analytes
Measurement of 17-hydroxyprogesterone is used in the diagnosis of the commonest form of congenital adrenal hyperplasia, 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 excess 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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