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# CORTISOL TESTING: an important tool for the diagnosis of adrenal disorders

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## Abstract

Cortisol is a hormone secreted by the adrenal cortex. It plays an important role in the stress response and in the metabolism of carbohydrates, lipids and proteins. Its measurement is indicated in the diagnosis of adrenal gland dysfunction, particularly when hyper- or hypocortisolism is suspected. Cortisol may be measured in blood, urine or saliva. It circulates in the blood in a free form and in a protein-bound form, and its concentration varies according to the time of sampling. This gives rise to several possible assay methods, which must be selected according to the type of sample, the form of cortisol being sought, as well as sensitivity and precision.

## Definition

Basal glucocorticoid release (outside of stress) follows a diurnal rhythm, with high levels in the morning and low levels in the evening. In response to psychological or physiological stress, the brain perceives the stressor and triggers a cascade signal that leads to the release of glucocorticoids and adrenaline by the adrenal gland. Activation of the hypothalamic–pituitary–adrenal axis leads to the release of cortisol by the adrenal cortex. Activation of the hypothalamic–pituitary–adrenal axis leads to the release of Corticotrophin-Releasing Hormone (CRH) by the paraventricular nucleus of the hypothalamus. CRH then reaches the anterior pituitary, where it stimulates the release of corticotrophin (ACTH). The binding of ACTH to receptors in the adrenal cortex induces the synthesis and release of glucocorticoids, foremost among them cortisol, into the systemic circulation. This release is dominated by a circadian cycle, corticotropic activity starting at around 3–4 a.m.: the blood cortisol concentration is at its highest in the morning between 6 and 8 a.m. (just before waking) and at its lowest in the evening, at bedtime. In the blood, only 10% of cortisol circulates free and constitutes the physiologically active fraction, while 90% is bound: 15% to albumin and 75% to a specific transport protein called transcortin or CBG (*cortisol binding globulin*).

## Indications for testing

Cortisol testing is therefore an important diagnostic tool for clinicians, and in particular for endocrinologists. It is recommended when hypercortisolism is suspected, as for example Cushing's syndrome, and when hypocortisolism is suspected, as for example in adrenal insufficiency.

- **Hypercortisolism (Cushing's syndromes)**
  Cushing's syndrome is recognised by obesity of the upper half of the body, a puffy red appearance of the face, stretch marks, hirsutism, arterial hypertension, spaniomenorrhoea or impotence.
- **Hypocortisolism: primary adrenal insufficiency (Addison's disease)**
  Addison's disease manifests as evening fatigue and malaise related to hypotension. The presence of a heterogeneous melanoderma, predominating on the skin folds, the scars and the exposed areas, confirms the diagnosis.

Apart from Cushing's syndrome and Addison's disease, other conditions may also be associated with dysfunction of cortisol synthesis (Table 1).

## Preanalytical conditions

- **Plasma cortisol**
  Serum and heparinised plasma are the matrices most often used for the assay. Fasting and a rest period of at least fifteen minutes before sampling are recommended. Sampling must be carried out at 8 a.m. or at midnight. Any exertion or stress before the test should be avoided (it is important to let the patient rest before sampling). After collection, send the sample to the laboratory as soon as possible, as it must be processed within the hour.
- **Urinary cortisol**
  Collect the 24-hour urine on acid, as cortisol is fragile in an alkaline medium. Measure urinary creatinine in order to check the validity of the urine collection.
- **Salivary cortisol**
  Collect 1 ml of saliva (at minimum) by asking the patient to salivate directly into a sterile container. Then transfer the sample into a hermetically sealed tube and send it to the laboratory.

**Table 1: Aetiologies of hyper- and hypocortisolism**

| **Hypercortisolism** |
|---|
| ACTH-dependent Cushing's syndromes:<br>– Cushing's disease;<br>– Paraneoplastic syndromes. |
| ACTH-independent Cushing's syndromes:<br>– Adrenal adenoma;<br>– Adrenocortical carcinoma;<br>– Micronodular hyperplasia. |
| **Hypocortisolism** |
| Peripheral (primary) adrenal insufficiency:<br>– Cortical atrophy (autoimmune);<br>– Tuberculosis;<br>– Adrenal haemorrhage or infarction;<br>– Metastatic invasion;<br>– Infiltration (sarcoidosis, amyloidosis);<br>– Infectious causes;<br>– Iatrogenic (surgery, radiotherapy, drugs);<br>– Congenital (enzyme blocks);<br>– Adrenoleukodystrophy. |
| Secondary adrenal insufficiency (corticotropic insufficiency):<br>– Pituitary surgery or radiotherapy;<br>– Pituitary or hypothalamic tumour;<br>– Sheehan's syndrome;<br>– Infiltrative lesions;<br>– Prolonged corticosteroid therapy. |

## Total cortisol assay

It generally consists in determining the two forms of circulating blood cortisol (free and bound). Total cortisol may be measured by several different methods, but immunoassay remains the most widely used method. The principle is based on competition between a labelled cortisol (bearing a luminescent structure) and the cortisol of the patient's sample for an antibody (polyclonal or monoclonal) directed against cortisol. These immunoassays are automated and allow rapid reporting of results, a high assay throughput and the use of a limited volume of plasma. In cases of hypercortisolism, certain complementary assays are recommended for the aetiological work-up. These are:

- Plasma ACTH measurement and possibly other pituitary peptides;
- Cortisol measurement carried out over a circadian cycle: for example, blood samples taken at 8 a.m., 12 p.m., 4 p.m. and 8 p.m. Indeed, the disappearance of the circadian cycle is one of the first signs of adrenal overactivity;
- Urinary free cortisol;
- Salivary cortisol.

These assays may also be performed during dynamic stimulation (Synacthen) or suppression (dexamethasone) tests.

Adrenal and/or pituitary exploration by imaging may also be necessary to complete the diagnosis.

Certain factors can modify the plasma cortisol concentration and thereby alter the value of the assay. This is the case with pregnancy or high-dose oestrogen treatment, which cause an increase in the hepatic synthesis of transcortin (CBG), generating hypercortisolaemia without any change in the free fraction. Blood cortisol is also increased in anorexia nervosa, acute alcohol intoxication, haemorrhagic or septic shock states, cirrhosis and endogenous depression. Finally, in obese patients, in whom a situation of functional (or training-related) hypercortisolism is observed.

## Free cortisol assay

**– Urinary cortisol**

The free fraction of cortisol (about 10%) constitutes the physiologically active fraction, but also the fraction rapidly metabolised by the liver or eliminated in the urine. About 1% of the daily cortisol production is found unchanged, unmetabolised, in the urine. Measurement of urinary free cortisol on a 24-hour diuresis is one of the essential elements in the positive diagnosis of Cushing's syndrome; however, it is important to carry out a preanalytical treatment of the sample in order to remove substances such as steroid metabolites that may interfere with the assay. This treatment requires technically difficult purification steps (dichloromethane extraction followed by chromatographic purification), suitable premises and experienced staff. When the sample pre-treatment phase is properly performed, the urinary assay allows precise quantification of free cortisol without overestimation due to interfering peaks. Urinary creatinine must be measured in parallel in order to ensure the quality of the urine collection.

Urinary cortisol is rarely increased in obese patients, unlike serum cortisol. However, it is sometimes increased outside of hypercortisolism in endogenous depression, in pancreatitis and following certain surgical procedures.

**– Salivary cortisol**

Cortisol may also be determined in saliva. Indeed, salivary cortisol represents the free, bioavailable form of blood cortisol. Results in saliva are not influenced by physiological or pathological variations in the secretion of the cortisol carrier protein, CBG. Various saliva collection systems exist but, too often, they are poorly understood or poorly used and the quantity of saliva is unusable. In general, at least 1 ml of saliva is needed in order to process the sample under good conditions. The assay must be ultra-sensitive and requires a concentration step in order to adapt the measurement range to the low salivary cortisol concentration.

## Reference values

| Blood cortisol: |
| --- |
| – 8 a.m.: 50 to 200 ng/ml (125 to 550 nmol/L)<br>– Midnight: 25 to 100 ng/ml (67 to 275 nmol/L)<br>– In children under 10 years of age: 50 to 150 ng/mL |
| **Urinary cortisol:** |
| 30 to 200 nmol/24h |
| **Salivary cortisol:** |
| • 8 a.m.: 10.5 to 27 nmol/L<br>• 12 p.m.: 6.6 to 13 nmol/L<br>• 4 p.m.: 5.2 to 9.2 nmol/L<br>• 8 p.m.: 1.2 to 6 nmol/L |

## References


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