Test Definition: CORTO

Cortisol, Free and Total, Serum

Overview

Useful For

Assessment of cortisol status in cases where there is known or a suspected abnormality in cortisol-binding proteins or albumin

Assessment of adrenal function in the critically ill or stressed patient, thus preventing unnecessary use of glucocorticoid therapy

Second-order testing when cortisol measurement by immunoassay (eg, CORT / Cortisol, Serum) gives results that are not consistent with clinical symptoms, or if patients are known to, or suspected of, taking exogenous synthetic steroids

An adjunct in the differential diagnosis of primary and secondary adrenal insufficiency

An adjunct in the differential diagnosis of Cushing syndrome

Profile Information

Test Id	Reporting Name	Available Separately	Always Performed
CINP	Cortisol, S, LC-MS/MS	Yes	Yes
CORTF	Cortisol, Free, S	Yes	Yes

Special Instructions

- Steroid Pathways

Method Name

Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

NY State Available

Yes

Specimen

Specimen Type

Serum Red

Ordering Guidance

For confirming the presence of synthetic steroids, order SGSS / Synthetic Glucocorticoid Screen, Serum.

Cushing syndrome is characterized by increased serum cortisol levels. However, the 24-hour urinary free cortisol

excretion is the preferred screening test for Cushing syndrome, specifically CORTU / Cortisol, Free, 24 Hour, Urine that utilizes liquid chromatography tandem mass spectrometry. A normal result makes the diagnosis unlikely.

The most common cause of increased plasma cortisol levels in women is a high circulating concentration of estrogen (ie, estrogen therapy, pregnancy) resulting in increased concentration of corticosteroid-binding globulin. This does not result in an increase in the free, bioactive cortisol fraction. For this reason, measurement of 24-hour urinary free cortisol (CORTU / Cortisol, Free, 24 Hour, Urine) or demonstration of absent diurnal variation (ie, by midnight salivary cortisol measurement SALCC / Cortisol and Cortisone, Saliva) are the preferred means of diagnosing spontaneous Cushing syndrome.

This test is not recommended for evaluating response to metyrapone; DCORT / 11-Deoxycortisol, Serum is more reliable.

A low plasma cortisol level does not give conclusive indication of congenital adrenal hyperplasia. DCORT / 11-Deoxycortisol, Serum; OHPG / 17-Hydroxyprogesterone, Serum; and DHEA_ / Dehydroepiandrosterone (DHEA), Serum provide a more accurate and specific determination of the enzyme deficiency.

Necessary Information

Include time of collection.

Specimen Required

Supplies: Sarstedt Aliquot Tube, 5 mL (T914)
Collection Container/Tube: Red top (serum gel/SST are **not acceptable**)
Submission Container/Tube: Plastic vial
Specimen Volume: 1.85 mL
Collection Instructions:
1. Morning (8 a.m.) specimens are preferred. The 8 a.m. cortisol can be referred to as the a.m. cortisol and can be collected anywhere between 6 a.m. and 10:30 a.m. in the morning.
2. Centrifuge and aliquot serum into a plastic vial.
Additional Information: If multiple specimens are collected, send separate order for each specimen.

Specimen Minimum Volume

1 mL

Reject Due To

Gross hemolysis	Reject
Gross lipemia	OK
Gross icterus	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Serum Red	Refrigerated (preferred)	28 days	
	Ambient	28 days	
	Frozen	28 days	

Clinical & Interpretive

Clinical Information

Cortisol, the main glucocorticoid (representing 75%-95% of the plasma corticoids), plays a critical role in glucose metabolism and in the body's response to stress. Both hypercortisolism (Cushing disease) and hypocortisolism (Addison disease) can cause disease. Cortisol is also used to treat skin disease, allergic disorders, respiratory system disease, inflammatory disorders, and nephrotic syndrome.

Cortisol levels are regulated by corticotropin (previously adrenocorticotropic hormone: ACTH), which is synthesized by the pituitary in response to corticotropin-releasing hormone (CRH). CRH is released in a cyclic fashion by the hypothalamus, resulting in diurnal peaks (6 a.m.-8 a.m.) and troughs (11 p.m.) in plasma ACTH and cortisol levels.

The majority of cortisol circulates bound to corticosteroid-binding globulin (CBG) and albumin. Normally, less than 5% of circulating cortisol is free (unbound). Only free cortisol can access the enzyme transporters in liver, kidney, and other tissues that mediate metabolic and excretory clearance.

Historically, measurements of free cortisol have been achieved from indirect means using a ratio known as the free cortisol index. This measurement takes into account the amount of total cortisol and CBG to give a percentage and, ultimately, absolute value of free cortisol. These methods do not consider the possibility variations in albumin levels. These calculations also rely on CBG, which can be lowered in critically ill patients despite normal adrenal function. Equilibrium dialysis best serves to separate free from bound cortisol without disrupting the bound fraction.

Pathological hypercortisolism due to endogenous or exogenous glucocorticoids is termed Cushing syndrome. Signs and symptoms of pathological hypercortisolism may include central obesity, hypertension, hyperglycemia, hirsutism, muscle weakness, and osteoporosis. However, these symptoms and signs are not specific for pathological hypercortisolism. Most individuals with some or all of the symptoms and signs will not suffer from Cushing syndrome.

When Cushing syndrome is present, the most common cause is iatrogenic, due to repeated or prolonged administration of, mostly, synthetic corticosteroids. Spontaneous Cushing syndrome is less common and results from either primary adrenal disease (adenoma, carcinoma, or nodular hyperplasia) or an excess of ACTH (from a pituitary tumor or an ectopic source). ACTH-dependent Cushing syndrome due to a pituitary corticotroph adenoma is the most frequently diagnosed subtype, commonly seen in women in the third through fifth decades of life. The onset is insidious and usually occurs 2 to 5 years before a clinical diagnosis is made.

Hypocortisolism most commonly presents with nonspecific lassitude, weakness, hypotension, and weight loss. Depending on the cause, hyperpigmentation may be present. More advanced cases and patients submitted to physical stress (ie, infection, spontaneous or surgical trauma) also may present with abdominal pain, hyponatremia, hyperkalemia, hypoglycemia, and in extreme cases, cardiovascular shock, and kidney failure.

The more common causes of hypocortisolism are:
Primary adrenal insufficiency:
-Addison disease
-Congenital adrenal hyperplasia, defects in enzymes involved in cortisol synthesis

Secondary adrenal insufficiency:
-Prior, prolonged corticosteroid therapy
-Pituitary insufficiency
-Hypothalamic insufficiency

For more information see Steroid Pathways.

Reference Values

FREE CORTISOL
6-10:30 a.m. Collection: 0.121-1.065 mcg/dL

TOTAL CORTISOL
5-25 mcg/dL (a.m.)
2-14 mcg/dL (p.m.)
Pediatric reference ranges are the same as adults, as confirmed by peer-reviewed literature.

Petersen KE. ACTH in normal children and children with pituitary and adrenal diseases. I. Measurement in plasma by radioimmunoassay-basal values. Acta Paediatr Scand. 1981;70(3):341-345

Interpretation

Cortisol is converted to cortisone in human kidneys and cortisone is less active toward the mineralocorticoid receptor. The conversion of cortisol to cortisone in the kidney is mediated by 11- beta-hydroxysteroid dehydrogenase isoform-2. Also, cortisol renal clearance will be reduced when there is a deficiency in the cytochrome P450 3A5 (CYP3A5) enzyme as well as a deficiency in P-glycoprotein.

Cortisol-binding globulin (CBG) has a low capacity and high affinity for cortisol, whereas albumin has a high capacity and low affinity for binding cortisol. Variations in CBG and serum albumin due to kidney or liver disease may have a major impact on free cortisol.

Based on the study by Bancos,(1) normal ranges of free cortisol found in patients without adrenal insufficiency were:
-Free cortisol at baseline: median 0.400 mcg/dL (interquartile range: IQR 2.5%-97.5%: 0.110-1.425 mcg/dL)
-Free cortisol at 30 minutes: median 1.355 mcg/dL (IQR 2.5%-97.5%: 0.885-2.440 mcg/dL)
-Free cortisol at 60 minutes: median 1.720 mcg/dL (IQR 2.5%-97.5%: 1.230-2.930 mcg/dL)

Based on the study by Bancos,(1) the following cutoffs were calculated for exclusion of adrenal insufficiency:
-Free cortisol at baseline*: greater than 0.271 mcg/dL (>271 ng/dL, area under the curve: AUC 0.81)
-Free cortisol at 30 minutes: greater than 0.873 mcg/dL (>873 ng/dL, AUC 0.99)
-Free cortisol at 60 minutes: greater than 1.190 mcg/dL (>1190 ng/dL, AUC 0.99)
(*note that baseline free cortisol should not be used to exclude adrenal insufficiency given low performance)

The use of free cortisol in the management of glucocorticoid levels in the stressed patient due to major surgery or trauma requires further studies to establish clinical dosing levels and efficacy.

Cortisol pediatric reference ranges are generally the same as adults as confirmed by peer-reviewed literature.(2)

In primary adrenal insufficiency, corticotropin (previously adrenocorticotropic hormone: ACTH) levels are increased and

cortisol levels are decreased; in secondary adrenal insufficiency both ACTH and cortisol levels are decreased.

When symptoms of glucocorticoid deficiency are present and the 8 a.m. plasma cortisol value is less than 10 mcg/dL (or the 24-hour urinary free cortisol value is <50 mcg/24 hours), additional studies are needed to establish the diagnosis. The 3 most frequently used tests are the ACTH (cosyntropin) stimulation test, the metyrapone test, and insulin-induced hypoglycemia test. First, the basal plasma ACTH concentration should be measured and the short cosyntropin stimulation test performed.

Symptoms or signs of Cushing syndrome in a patient with low serum and urine cortisol levels suggest possible exogenous synthetic steroid effects.

Cautions

Cortisol levels may be increased in pregnancy and with exogenous estrogens. Use of the antineoplastic drug mitotane also increases cortisol-binding globulin and total cortisol.

When cortisol assays are used for serial monitoring, the same methodology should be used throughout.

There is little, if any, value in an isolated p.m. serum cortisol measurement.

Acute stress (including hospitalization and surgery), alcoholism, depression, and many drugs (ie, exogenous cortisones, anticonvulsants) can obliterate normal diurnal variation, affect response to suppression/stimulation tests, and cause elevated baseline levels.

Clinical Reference

1. Bancos I, Erickson D, Bryant S, et al. Performance of free versus total cortisol following cosyntropin stimulation testing in an outpatient setting. Endocr Pract. 2015;21(12):1353-1363. doi:10.4158/EP15820.OR
2. Petersen KE. ACTH in normal children and children with pituitary and adrenal diseases. I. Measurement in plasma by radioimmunoassay-basal values. Acta Paediatr Scand. 1981;70(3):341-345. doi:10.1111/j.1651-2227.1981.tb16561.x
3. Petersen KE. ACTH in normal children and children with pituitary and adrenal diseases. I. Measurement in plasma by radioimmunoassay-basal values. Acta Paediatr Scand. 1981;70(3):341-345. doi:10.1111/j.1651-2227.1981.tb16561.x
4. Ho JT, Al-Musalhi H, Chapman MJ, et al. Septic shock and sepsis: a comparison of total and free plasma cortisol levels. J Clin Endocrinol Metab. 2006;91(1):105-114. doi:10.1210/jc.2005-0265
5. le Roux CW, Chapman GA, Kong WM, Dhillo WS, Jones J, Alaghband-Zadeh J. Free cortisol index is better than serum total cortisol in determining hypothalamic-pituitary-adrenal status in patients undergoing surgery. J Clin Endocrinol Metab. 2003;88(5):2045-2048. doi:10.1210/jc.2002-021532
6. Huang W, Kalhorn TF, Baillie M, Shen DD, Thummel KE. Determination of free and total cortisol in plasma and urine by liquid chromatography-tandem mass spectrometry. Ther Drug Monit. 2007;29(2):215-224. doi:10.1097/FTD.0b013e31803d14c0

Performance

Method Description

Total Cortisol:
High-performance liquid chromatography (HPLC) with triple quad mass spectrometer (LC-MS/MS) Deuterated stable

isotopes (d4-cortisol, d7-androstenedione, d8 17-hydroxyprogesterone) are added to a 0.1 mL serum sample as internal standards. Cortisol, androstenedione, 17-hydroxyprogesterone and the internal standards are extracted using online turbulent flow HPLC extraction and are analyzed by LC-MS/MS.(Unpublished Mayo method)

Free Cortisol:
To measure free cortisol in serum, it is vital to separate the unbound from its conjugated form without disrupting the equilibrium of the sample. The rapid equilibrium dialysis (RED) plate is used to perform a simple means of equilibrium dialysis in a 96-well format. Sample is placed into one of two chambers separated by vertical cylinder of equilibrium membrane (MWCO 8,000). The second chamber is filled with dialysis buffer. Both chambers reside in a polypropylene plate with standard 96-well format. After gently shaking overnight at 37 degrees C, the free cortisol is at equilibrium within the dialysis chamber. The dialysate is removed and d3-cortisol is added as an internal standard. The dialysate mixture is then analyzed using turbo flow liquid chromatography combined with a heated nebulizer ion source and tandem mass spectrometry.(Unpublished Mayo method)

PDF Report
No

Day(s) Performed
Tuesday, Thursday, Friday

Report Available
3 to 11 days

Specimen Retention Time
14 days

Performing Laboratory Location
Mayo Clinic Laboratories - Rochester Superior Drive

Fees & Codes

Fees
- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact Customer Service.

Test Classification
This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. It has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information
82530
82533

LOINC® Information

Test Definition: CORTO

Cortisol, Free and Total, Serum

Test ID	Test Order Name	Order LOINC® Value
CORTO	Cortisol, Free and Total, S	100662-6

Result ID	Test Result Name	Result LOINC® Value
84279	Cortisol, S, LC-MS/MS	2143-6
23606	AM Cortisol	9813-7
23607	PM Cortisol	9812-9
65423	Cortisol, Free, S	2145-1