Test Definition: CORTF

Cortisol, Free, 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

Method Name

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

NY State Available

Yes

Specimen

Specimen Type

Serum Red

Necessary Information

Include time of collection.

Specimen Required

Supplies: Sarstedt Aliquot Tube, 5 mL (T914)
Collection Container/Tube: Red top
Submission Container/Tube: Plastic vial
Specimen Volume: 1.25 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 any time 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 a 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 take into account the possible 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.

Reference Values

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

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 renal 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)
*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.

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.

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. Hamrahian AH, et al. Measurements of Serum Free Cortisol in Critically Ill Patients. New England Journal of Medicine 2004;350;16:1629-1638
3. 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
4. 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

Performance

Method Description

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. The dialysate mixture is then analyzed using turbo flow liquid chromatography combined with a heated nebulizer ion source (APCI) and tandem mass spectrometry. (Unpublished Mayo method)

PDF Report

No

Test Definition: CORTF

Cortisol, Free, Serum

Day(s) Performed
Tuesday, Thursday, Friday

Report Available
2 to 11 days

Specimen Retention Time
2 weeks

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

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
CORTF	Cortisol, Free, S	2145-1

Result ID	Test Result Name	Result LOINC® Value
65423	Cortisol, Free, S	2145-1