Test Definition: SALCC

Cortisol and Cortisone, Saliva

Overview

Useful For

Screening and diagnosis of Cushing syndrome/hypercortisolism

Adjunctive evaluation of adrenal insufficiency (using waking salivary cortisone/cortisol)

May be useful to assess for hypercortisolism/other adrenal disorders during pregnancy/oral estrogen therapy (when total cortisol is increased due to increase in cortisol-binding globulin) or in states of low cortisol-binding globulin (critical illness)

Evaluation of suspected contamination from topical steroids

Special Instructions

- Cortisol and Cortisone - Saliva Collection Instructions

Method Name

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

NY State Available

Yes

Specimen

Specimen Type

Saliva

Necessary Information

Collection time is required.

Specimen Required

If multiple specimens are collected, submit each Salivette under a separate order number.

Patient Preparation:

1. Patient **should not** brush teeth, eat, drink, or take any oral medication for at least 60 minutes before specimen collection.
2. Have patient rinse mouth thoroughly with water for 1 to 5 seconds. Wait 10 minutes before collecting the specimen to avoid contamination of the saliva by an interfering substance.

Supplies: Cortisol, Saliva Collection Kit (T514)
Container/Tube: Sarstedt Salivette
Specimen Volume: 1.5 mL

Collection Instructions:
1. Provide patient with a Saliva Collection Kit (Salivette) containing the Cortisol and Cortisone - Saliva Collection Instructions and ask them to follow the instructions as written.
2. If performing a late-night salivary cortisol (LNSC) test, specimen should be collected immediately prior to going to bed. If patient is undergoing assessment for adrenal insufficiency, specimen should be collected immediately after waking up. The patient should follow their normal sleeping schedule. Record collection time on the Cortisol and Cortisone - Saliva Collection Instructions sheet.
3. Instruct patient to return Cortisol and Cortisone - Saliva Collection Instructions with the appropriately labeled Salivette to the laboratory.
Additional Information: Reference intervals are available for LNSC testing (immediately before going to bed) and immediately after waking for assessment of adrenal insufficiency.

Specimen Minimum Volume
0.6 mL

Reject Due To

All specimens will be evaluated at Mayo Clinic Laboratories for test suitability.

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Saliva	Refrigerated (preferred)	28 days	
	Ambient	14 days	
	Frozen	60 days	

Clinical & Interpretive

Clinical Information
Cortisol secretion is regulated through the hypothalamic-pituitary-adrenal axis. The diagnosis of both Cushing syndrome (CS) and adrenal insufficiency (AI) relies largely on cortisol measurements. CS results from chronic endogenous glucocorticoid excess and may be pituitary, ectopic, or adrenal in origin. In contrast, AI reflects cortisol deficiency that may be primary or central.

Cortisol follows a circadian rhythm, with peak levels in the early morning and a nadir around midnight. In most patients with CS, this circadian pattern is disrupted, with inappropriately elevated late-night cortisol concentrations. Measurement of late-night salivary cortisol (LNSC), collected immediately prior to going to bed, is a recommended first-line diagnostic test for hypercortisolism. Conversely, measurement of cortisol upon waking is a first line test for AI.

Salivary cortisol testing offers several advantages: it is noninvasive, stable at room temperature, and reflects the free, biologically active cortisol fraction. In the salivary glands, cortisol is rapidly converted to cortisone via 11beta-hydroxysteroid dehydrogenase type 2. Because salivary cortisol can be affected by contamination (eg, topical hydrocortisone, gingival bleeding), salivary cortisone is often a more reliable marker of endogenous cortisol production. Studies suggest that salivary cortisone performs as well or better than salivary cortisol in detecting hypercortisolism. As such, measuring salivary cortisone in addition to cortisol may improve diagnostic accuracy, reduce indeterminate results,

and help identify contamination. Emerging evidence also supports the use of waking salivary cortisol and cortisone measurements to diagnose or exclude AI, potentially reducing the need for dynamic testing and offering greater convenience for patients.

Reference Values

Cortisol:
Immediately after waking: 85-620 ng/dL
Immediately prior to going to bed: <127 ng/dL

Cortisone:
Immediately after waking: 510-2110 ng/dL
Immediately prior to going to bed: 62-624 ng/dL

Cortisol/Cortisone Ratio interpretation:
Cortisol should be lower than cortisone in saliva. A ratio greater than 1 suggests contamination with blood or exogenous cortisol. A ratio greater than 2 indicates contamination is likely. In these cases, recollection is recommended.

Interpretation

Cushing syndrome/hypercortisolism:
Both salivary cortisol and cortisone should be low at night. In Cushing syndrome (CS), late night concentrations of both analytes are typically elevated due to loss of the normal circadian nadir. Diagnosis typically requires concordant evidence from additional tests, such as overnight dexamethasone suppression testing and 24-hour urine cortisol assessment.

Late night salivary cortisone offers improved sensitivity and specificity compared to salivary cortisol.

Late-night salivary cortisol levels above 110-145 ng/dL are considered abnormal, suggestive of hypercortisolism.

Late night salivary cortisone levels above 487-649 ng/dL are considered abnormal, suggestive of hypercortisolism.

Adrenal insufficiency:
Waking salivary cortisone and/or cortisol measurements can help exclude or suggest adrenal insufficiency (AI) and may reduce the need for dynamic testing

Based on the 2023 study by Debono et al*:
Waking salivary cortisone greater than 612 ng/dL excludes AI
Waking salivary cortisol greater than 180 ng/dL excludes AI
Waking salivary cortisone less than 251 ng/dL diagnoses AI
Waking salivary cortisol less than 25 ng/dL diagnoses AI

*In NEJM study by Debono et al from 2023, the area under the curve (AUC) of waking salivary cortisone was 0.95. A cutoff of greater than 612 ng/dL had a sensitivity of 97%, specificity of 67%, and a negative predictive value (NPV) of 96% for excluding adrenal insufficiency. A cutoff of less than251 ng/dL had a sensitivity of 76%, specificity of 97%, and positive predictive value (PPV) of 95% in confirming adrenal insufficiency. In the same study, salivary cortisol performed worse (AUC of 0.89), with cutoff of >180 ng/dL having NPV of 94% and <25 ng/dL - PPV of 93%.

In addition, based on the in-house study of 640 patients with paired venipuncture-based serum cortisol and salivary cortisol and cortisone, corresponding serum cortisol - salivary cortisol and cortisone thresholds are listed below.

Table. Salivary steroid thresholds corresponding to serum steroid measurements

Serum cortisol cutoff mcg/dL	Saliva steroids ng/dL	Area under the curve (95% CI)	Optimized cutoff	Rule-out threshold (95% sensitivity)	Rule-in threshold (95% specificity)
< or =1.8	Cortisone	0.98 (0.96-0.99)	117	102	122
	Cortisol	0.91 (0.89-0.94)	17	<10	32
< or =5	Cortisone	0.99 (0.98-1.00)	327	300	332
	Cortisol	0.99 (0.98-0.99)	36	35	44
> or =10	Cortisone	0.99 (0.99-1.00)	650	375	822
	Cortisol	0.99 (0.99-1.00)	83	50	190
> or =18	Cortisone	0.99 (0.99-1.00)	1232	780	1447
	Cortisol	0.99 (0.99-1.00)	378	173	456

Cortisol/cortisone ratio greater than 1 is suggestive of contamination.

Cautions

Several factors may influence results:
-Stress, acute illness, psychiatric conditions, alcohol use, and certain medications can elevate levels
-Timing of collection is critical due to circadian variation
-Irregular sleep schedules (eg, shift work) may affect interpretation
-Topical hydrocortisone may falsely elevate salivary cortisol, whereas cortisone is less affected

Clinical Reference

1. Vaidya A, Findling J, Bancos I. Adrenal insufficiency in adults: A review. JAMA. 2025;334(8):714-725
2. Berndt V, Dahlqvist P, de Verdier J, Ryberg H, Ragnarsson O. The diagnostic value of salivary cortisol and salivary cortisone in patients with suspected hypercortisolism. Front Endocrinol (Lausanne). 2022;13:1028804
3. Backlund N, Brattsand G, Israelsson M, et al. Reference intervals of salivary cortisol and cortisone and their diagnostic accuracy in Cushing's syndrome. Eur J Endocrinol. 2020;182(6):569-582
4. Lages AS, Frade JG, Oliveira D, et al. Late-night salivary cortisol: cut-off definition and diagnostic accuracy for Cushing's syndrome in a portuguese population. Acta Med Port. 2019;32(5):381-387
5. Ponzetto F, Settanni F, Parasiliti-Caprino M, et al. Reference ranges of late-night salivary cortisol and cortisone measured by LC-MS/MS and accuracy for the diagnosis of Cushing's syndrome. J Endocrinol Invest. 2020;43(12):1797-1806

6. van Baal L, Wichert M, Zwanziger D, et al. Distinct Late-Night Salivary Cortisol Cut-Off Values for the Diagnosis of Hypercortisolism. Horm Metab Res. 2021;53(10):662-671
7. Debono M, Elder CJ, Lewis J, et al. Home waking salivary cortisone to screen for adrenal insufficiency. NEJM Evid. 2023.2(2):p.EVIDoa2200182
8. Fleseriu M, Auchus R, Bancos I, et al. Consensus on diagnosis and management of Cushing's disease: a guideline update. Lancet Diabetes Endocrinol. 2021;9(12):847-875
9. Mohamed RS, Abuelgasim B, Barker S, et al. Late-night salivary cortisol and cortisone should be the initial screening test for Cushing's syndrome. Endocr Connect. 2022;11(7):e220050
10. Debono M, Caunt S, Elder C, et al. Real world evidence supports waking salivary cortisone as a screening test for adrenal insufficiency. Clin Endocrinol (Oxf). 2023;99(6):517-524
11. Kvam Hellan K, Lyngstad M, Methlie P, Lovas K, Husebye ES, Ueland GA. Utility of salivary cortisol and cortisone in the diagnostics of adrenal insufficiency. J Clin Endocrinol Metab, 2025.110(5):1218-1223
12. Langelaan MLP, Kisters JMH, Oosterwerff MM, Boer AK. Salivary cortisol in the diagnosis of adrenal insufficiency: cost efficient and patient friendly. Endocr Connect. 2018;7(4):560-566

Performance

Method Description
Isotopically labeled cortisol and cortisone are added to the sample as internal standards. Cortisol, cortisone, and coinciding internal standards are extracted from the specimens using online solid phase extraction (SPE) followed by liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis.(Unpublished Mayo method)

PDF Report
No

Day(s) Performed
Monday through Friday

Report Available
3 to 6 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

82533 - Cortisol
82542 - Cortisone

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
SALCC	Cortisol and Cortisone, Saliva	In Process

Result ID	Test Result Name	Result LOINC® Value
623629	Cortisol Saliva	2142-8
623630	Cortisone Saliva	In Process
623631	Cortisol/Cortisone ratio	In Process