

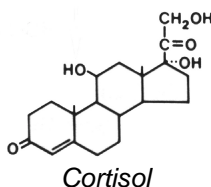
Salivary Cortisol Technical Issues

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General Information

Cortisol is a lipophilic steroid with low molecular weight (MW ~362 Dalton). Following ACTH binding to membrane receptors on cells of the adrenal cortex, cortisol is synthesized and released into the blood stream. Up to 95% of the secreted cortisol will be bound to large proteins (CBG, albumin) and carried throughout the body in the blood. Since the vast majority of cortisol actions rely on binding to its cytosolic mineralocorticoid and glucocorticoid receptors, only the small fraction of unbound, i.e., free cortisol is thought to be biologically active. Due to its low molecular weight and lipophilic nature, unbound cortisol enters cells by passive diffusion which makes it feasible to measure the free cortisol fraction in all bodily fluids.

While the assessment of cortisol in sweat or tears is only of theoretical importance and urinary cortisol of decreasing interest, salivary cortisol has become an invaluable tool for both basic scientists and clinicians. A number of significant advantages over the assessment of cortisol in blood has resulted in an steadily increasing interest in salivary cortisol.



FAQs (frequently asked questions) about salivary cortisol

Q: *Does the concentration of cortisol in saliva accurately reflect the level in blood?*

A: Yes, when compared to the amount of unbound cortisol in serum or plasma samples. A number of studies have revealed correlations between the two specimen of $r > 0.90$. However, the correlation between the total cortisol levels in blood and salivary cortisol is usually weaker due to different amounts of CBG found in blood (e.g., high levels of CBG due to oral contraceptive use results in high total cortisol levels; free cortisol levels are usually unaltered in states of increased CBG).

Q: *Does the salivary cortisol levels depend on saliva flow rate?*

A: No. Studies looking at salivary cortisol levels obtained under minimal and maximal flow rate clearly showed that the cortisol levels in saliva do not depend on flow rate.

Q: *How can I obtain a saliva samples for cortisol analysis?*

A: There are a number of ways to sample saliva. One of the easiest is to use a device called the "Salivette". The Salivette is manufactured and sold by Sarstedt (phone number for Germany: 02293-3050; for the U.S.: 704-465-4000). Subjects should be instructed to gently chew on the cotton roll to stimulate saliva flow rate. With this it usually takes less than a minute to obtain a saliva sample of 0.5-1 ml volume.



The Salivette

Q: *Which medications or food ingredients will interfere with salivary cortisol?*

A: The level of salivary cortisol is influenced by drugs such as prednisone, dexamethasone and other steroids administered orally or i.v. While prednisone usually crossreacts with the antiserum used for assaying cortisol (leading to false high values), dexamethasone will significantly suppress the HPA axis (resulting in low cortisol levels). Furthermore, if the subjects deliver saliva samples with a low pH (e.g., following stimulation of saliva flow by crystals of vitamin C, consumption of fruit juices immediately before sampling etc.) most immunoassay will produce false high cortisol levels.

Q: *Do I have to store the saliva samples in the refrigerator or freezer immediately?*

A: No. Studies have shown that salivary cortisol is stable at room temperature for a period of 2-4 weeks. This means, that even after four weeks left without refrigeration a saliva sample will give the same cortisol level as with immediate determination or with the samples frozen until assay. Note however, that sampling devices like the Salivette will start to mold within 4-7 days at room temperature. This produces a very bad smelling sample. It does not appear to affect the cortisol levels, but it sure affects the mood of the technicians assaying the samples! So whenever possible, saliva samples should be stored at -20 degrees Celsius or lower.

Q: *Do I have to use dry ice if I send my samples to the laboratory?*

A: No. There is no refrigeration required for the transport between the subject's / patient's home and the lab (or sending a larger batch of samples from lab to lab). However, if it takes more than 4 days before the samples are delivered to the lab, dry ice will prevent the samples from molding.

Q: *Does repeated freezing and thawing of the saliva samples affect the cortisol levels?*

A: No.

Q: *How can I have my saliva samples assayed for cortisol?*

A: There are different options available today. First, several diagnostic companies now offer commercial assay kits for cortisol determination in saliva. Three of the better assays appear to be sold by (a) DSL (www.DSLabs.com), (b) Salimetrics (www.salimetrics.com) and (c) Orion (www.orion.com) These assays appear to give correct values with acceptable sample volumes required.

If you do not have a lab or the time to run the assays yourself, you can also send your samples to a lab which provide assay service. Besides our own lab in Düsseldorf, there are other research labs as well as a commercial lab which run custom assays.

Q: *How can I find out how accurate/reliable a lab is?*

A: One way to check this out is to split a saliva sample into several aliquots and do so with 3-10 samples of (possibly) different cortisol concentrations. Send 3-5 aliquots of each sample to the lab which you want to check (you have to give the different aliquots different I.D.s, of course). Divide the mean cortisol value (from the different aliquots) of each sample by the standard deviation and multiply the result by 100%. This will give you the intraassay coefficient of variation (c.v.) for the different samples. The c.v. should not exceed 12-15% (the lower the cortisol concentration, the higher the c.v. usually - rule of thumb).

Q: *What are the assay costs for a salivary cortisol determination?*

A: If you decide to purchase a kit and do the assays in your own lab, the price differs widely and depends on the conditions you obtain from the manufacturer. As a rule of thumb, expect to pay 3-5 USD per duplicate analysis (including standards and controls) for the reagents. If you send your samples to a lab which offers assays service, expect to pay between 2.50 - 15 USD for the same analysis.

(More detailed technical information can be obtained from our scientific papers. Please consult our website at www.uni-duesseldorf.de/~ck/index.html for publications. Most papers are available as PDF documents to be downloaded from our webpages.)