DRG

CE

DRG® Salivary Cortisol ELISA (SLV-2930)

IVD

Revised 7 April 2014 rm (Vers. 9.1)

INTRODUCTION

Intended Use

An enzyme immunoassay for the quantitative *in vitro diagnostic* measurement of active free cortisol (hydrocortisone and hydroxycorticosterone) in saliva. Measurements of cortisol are used in the diagnosis and treatment of disorders of the adrenal gland.

Summary and Explanation

The hormone Cortisol is vital for several functions of the human body. A strong correlation exists between stress related conditions and Cortisol levels (*1–3*) Cortisol is a steroid hormone made in the adrenal glands. Among its important functions in the body include roles in the regulation of blood pressure and cardiovascular function as well as regulation of the body's use of proteins, carbohydrates, and fats. Cortisol secretion increases in response to any stress in the body, whether physical (such as illness, trauma, surgery, or temperature extremes) or psychological. When cortisol is secreted, it causes a breakdown of muscle protein, leading to release of amino acids into the bloodstream. These amino acids are then used by the liver to synthesize glucose for energy, in a process called gluconeogenesis. This process raises the blood sugar level so the brain will have more glucose for energy. Cortisol also leads to the release of so-called fatty acids, an energy source from fat cells, for use by the muscles. Taken together, these energy-directing processes prepare the individual to deal with stressors and ensure that the brain receives adequate energy sources (*4*).

Cortisol is the most potent glucocorticoid produced by the human adrenal (*5-7*). It is synthesized from cholesterol and its production is stimulated by pituitary adrenocorticotropic hormone (ACTH) which is regulated by corticotropin releasing factor (CRF). ACTH and CRF secretions are inhibited by high cortisol levels in a negative feedback loop. Cortisol acts through specific intracellular receptors and affects numerous physiologic systems including immune function, glucose counter regulation, vascular tone, and bone metabolism.

Elevated cortisol levels and lack of diurnal variation have been identified with Cushing's disease (ACTH hypersecretion). Elevated circulating cortisol levels have also been identified in patients with adrenal tumors. Low cortisol levels are found in primary adrenal insufficiency (e.g. adrenal hypoplasia, Addison's disease) and in ACTH deficiency. Due to the normal circadian variation in cortisol levels (*8*), distinguishing normal from abnormally low cortisol levels can be difficult, therefore several daily collections are recommended.

Saliva is an excellent medium to measure steroids because it is a natural ultra-filtrate of blood, and steroids not bound by carrier proteins in the blood freely diffuse into saliva. Only about 1-10% of the steroids in blood are in the unbound or free form, and it is this fraction that diffuses into target tissues of the body, and into saliva (*9, 10*). The majority (90-99%) of steroid hormones in the blood are bound to carrier proteins (cortisol binding globulin, sex-hormone binding globulin and albumin) and are unavailable to target tissues. The process of passive diffusion of non-bound (free) steroid hormones occurs because these small molecules are of a low molecular weight (less than 400 daltons) and are relatively nonpolar, thus enabling them to freely diffuse from blood to saliva. Bound steroids are too large to diffuse freely through the salivary cells into the salivary gland lumen. (*11-14*)

PRINCIPLE OF THE TEST

The **DRG Salivary Cortisol ELISA KIT** is based on the competition principle and the microplate separation. An unknown amount of Cortisol present in the sample and a fixed amount of Cortisol conjugated with horse-radish peroxidase compete for the binding sites of mouse monoclonal Cortisol -antiserum coated onto the wells. After one hour incubation the microplate is washed to stop the competition reaction. After addition of the substrate solution the concentration of Cortisol is inversely proportional to the optical density measured.

Revised 7 April 2014 rm (Vers. 9.1)

WARNINGS AND PRECAUTIONS

1. This kit is for in vitro diagnostic use only. For professional use only.
2. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
3. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
4. The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. Allow the reagents to reach room temperature (21 °C - 26 °C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
12. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
16. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
17. Avoid contact with *Stop Solution* containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
18. Some reagents contain Proclin, BND and MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
21. For information on hazardous substances included in the kit please refer to Safety Data Sheets.
    Safety Data Sheets for this product are available upon request directly from DRG.

Revised 7 April 2014 rm (Vers. 9.1)

REAGENTS

Reagents provided

a. ***Microtiterwells***, 12x8 (break apart) strips, 96 wells;
coated with (mouse) anti-Cortisol antiserum.

b. ***Standard (Standard 0-6)***, 7 vials, 1 mL each, ready to use;
Concentrations: 0.0 – 2 – 5 – 10 – 20 – 40 - 80 ng/mL
Conversion factor: 1 pg/mL = 2.76 pmol/L.
Contain non-mercury preservative.

c. ***Control low / Control high***, 2 vials, 1.0 mL each, ready to use;
For control values and ranges please refer to vial label or QC-Datasheet.
Contain non-mercury preservative.

d. ***Enzyme Conjugate***, 1 vial, 26 mL, ready to use;
Cortisol conjugated to horseradish peroxidase.
Contain non-mercury preservative.

e. ***Substrate Solution*** 1 vial, 25 mL, ready to use;
Tetramethylbenzidine (TMB).

f. ***Stop Solution***, 1 vial, 14 mL, ready to use;
contains 0.5M H_2SO_4.
Avoid contact with the stop solution. It may cause skin irritations and burns.

g. ***Wash Solution***, 1 vial, 30 mL (40X concentrated);
Concentrate for 1200 mL.
see „Preparation of Reagents“.

Note: Additional *Standard 0* for sample dilution is available upon request (Cat. number SLV-2930-0STD).

Materials required but not provided

1. Calibrated EIA reader adjusted to read at 450 nm
2. Precision pipettes (100 and 200 µL)
3. Distilled or Deionized water
4. Timer (60 min. range)
5. Reservoirs (disposable)
6. Test tube or microtube rack in a microplate configuration
7. Linear-linear graph paper or software for data reduction

Storage Conditions

When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.
Opened kits retain activity for two month if stored as described above.

Revised 7 April 2014 rm (Vers. 9.1)

Reagent Preparation

Bring all reagents and required number of strips to room temperature prior to use.

Wash Solution

Add deionized water to the 40X concentrated Wash Solution.
Dilute 30 mL of concentrated *Wash Solution* with 1170 mL deionized water to a final volume of 1200 mL.
The diluted Wash Solution is stable for 2 weeks at room temperature.

Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Safety Data Sheet.

Damaged Test Kits

In case of any severe damage to the test kit or components, DRG has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

SPECIMEN Collection and Preparation

Eating, drinking, chewing gums or brushing teeth should be avoided for 30 minutes before sampling. Otherwise, it is recommended to rinse mouth thoroughly with cold water 5 minutes prior to sampling.

Do not collect samples when oral diseases, inflammation or lesions exist (blood contamination).

If there is visible blood contamination the patient specimen, it should be discarded, rinse the sampling device with water, wait for 10 minutes and take a new sample.

Note: Samples containing sodium azide should not be used in the assay.

Specimen Collection

Saliva samples should be collected only using special saliva sampling devices (vial and straw),
e.g. SALI-TUBES 100 (SLV-4158) or Salivette (Sarstedt cat.# 51.1534).

Due to the cyclic secretion pattern of steroid hormones it is important to care for a proper timing of the sampling.
In order to avoid arbitrary results we recommend that 5 samples always be taken within a period of
2 – 3 hours (*multiple sampling*) preferably before a meal.
As food might contain significant amounts of steroid hormones samples preferably should be taken while fasting. If fasting should be a problem the collection period should be timed just before lunch or before dinner.

Specimen Storage and Preparation

The saliva samples may be stored at 2 °C to 8 °C up to one week, and should be frozen at –20 °C for longer periods; repeated thawing and freezing is no problem.

Each sample has to be frozen, thawed, and centrifuged at least once in order to separate the mucins by centrifugation.
Upon arrival of the samples in the lab the samples have to stay in the deep freeze at least overnight. Next morning the frozen samples are warmed up to room temperature and mixed carefully.
Then the samples have to be centrifuged for 5 to 10 minutes (at 3000 - 2000 x g).

Revised 7 April 2014 rm (Vers. 9.1)

Now the clear colorless supernatant is easy to pipette.

If a set of multiple samples is to be tested, the lab (after at least one freezing, thawing, and centrifugation cycle) has to mix the 5 single samples in a separate sampling device and perform the testing from this mixture.

Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with *Standard 0* and re-assayed as described in Assay Procedure.
For the calculation of the concentrations this dilution factor has to be taken into account.
Example:
a) Dilution 1:10: 10 µl saliva + 90 µl *Standard 0* (mix thoroughly)
b) Dilution 1:100: 10 µl of dilution a) + 90 µl *Standard 0* (mix thoroughly).

ASSAY PROCEDURE

General Remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.

Test Procedure

Each run must include a standard curve.

1. Secure the desired number of coated strips in the frame holder.
2. Dispense **100 µL** of each Cortisol *Standard* and *Control* into appropriate wells.
3. Dispense **100 µL** of each sample into selected wells.
4. Dispense **200 µL** of *Enzyme Conjugate* into each sample and standard well and mix the plate for thoroughly for 10 seconds.
5. Incubate for **60 minutes** at room temperature.
6. Briskly shake out the contents of the wells and rinse the wells 3 times with diluted Wash Solution (400 µL per well). Strike the inverted wells sharply on absorbent paper towel to remove residual droplets.
7. Add **200 µL** of *Substrate Solution* to each well.
8. Incubate for **30 minutes** at room temperature.
9. Stop the reaction by adding **100 µL** of *Stop Solution* to each well.

10. Determine the absorbance of each well at 450 ± 10 nm.
    It is recommended that the wells be read within 10 minutes.

Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred calculation method. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 80 ng/mL. For the calculation of the concentrations this dilution factor has to be taken into account.

Example of Typical Standard Curve

The following data is for demonstration only and **cannot** be used in place of data generations at the time of assay.

Standard	Absorbance Units (450 nm)
Standard 0 (0 ng/mL)	1.88
Standard 1 (2 ng/mL)	1.75
Standard 2 (5 ng/mL)	1.58
Standard 3 (10 ng/mL)	1.39
Standard 4 (20 ng/mL)	1.09
Standard 5 (40 ng/mL)	0.75
Standard 6 (80 ng/mL)	0.47

EXPECTED NORMAL VALUES

In order to determine the normal range of SLV cortisol, 109 saliva samples from adult male and female apparently healthy subjects, ages 20 to 80 years, were collected in the morning and analyzed using the DRG SLV Cortisol ELISA kit. The following range was calculated from this study.

Adults: 0.12 – 1.47 µg/dL or 1.2 – 14.7 ng/mL

The results alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests. Since cortisol levels show diurnal cycles, we recommend that the samples be obtained the same hour each day. Furthermore, we recommend that each laboratory determine its own range for the population tested.

Revised 7 April 2014 rm (Vers. 9.1)

Quality Control

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.

The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.

It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.

Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid.

In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.

After checking the above mentioned items without finding any error contact your distributor or DRG directly.

PERFORMANCE CHARACTERISTICS

Assay Dynamic Range

The range of the assay is between 0.537 – 80 ng/mL.

Specificity

The following materials have been evaluated for cross reactivity. The percentage indicates cross reactivity at 50% displacement compared to Cortisol.

Steroid	% Cross reaction
Cortisol	100
Corticosterone	29.00
Cortisone	3.00
11-Deoxycortisol	< 1.00
17-OH Progesterone	< 0.50
Prednisone	<0.10
Progesterone	< 0.10
Dexamethazone	< 0.10
Desoxycorticosterone	< 0.10
Dehydroepiandrosterone sulfate	< 0.10
Estradiol	< 0.10
Estriol	< 0.10
Estrone	< 0.10
Testosterone	< 0.10

Sensitivity

The lowest detectable level of Cortisol that can be distinguished from the Zero Standard is 0.537 ng/mL or 0.0537 µg/dL at the 95 % confidence limit.

Reproducibility

Intra-Assay

The intra-assay variation was determined by replicate measurements of 4 saliva samples using DRG ELISA kit. The within assay variability is shown below:

Mean (ng/mL)	4.52	0.94	12.79	17.50
SD (ng/mL)	0.120	0.042	0.230	0.258
CV (%)	2.65	4.52	1.80	1.47
n =	20	20	20	20

Inter-Assay

The inter-assay (between-run) variation was determined by quadruplicate measurements of commercial control samples in three different days runs.

Mean (ng/mL)	24.29	40.85
SD (ng/mL)	1.81	2.38
CV (%)	7.47	5.82
n =	12	12

Inter-Lot

The Inter-Lot (between-lot) variation was determined by duplicate measurements of five saliva samples in three different kit lots. The between run variability is shown below:

Mean (ng/mL)	1.22	12.65	15.81	4.16	4.53
SD (ng/mL)	0.07	0.35	0.70	0.10	0.12
CV(%)	5.97	2.73	4.43	2.35	2.72
N =	9	9	9	9	9

Recovery

Recovery of the DRG ELISA was determined by adding increasing amounts of the analyte to three different saliva samples containing different amounts of endogenous analyte. Each sample (non-spiked and spiked) was assayed and analyte concentrations of the samples were calculated from the standard curve. The percentage recoveries were determined by comparing expected and measured values of the samples

Sample	Endogenous cortisol ng/mL	Added cortisol ng/mL	Measured OD mean of duplicate (450 nm)	Measured Conc. SLV cortisol ng/mL	Expected conc ng/mL	Recovery (%)
1	0.90	0.00	1.284	0.90		
		40.00	0.175	38.74	40.90	94.7
		20.00	0.262	22.45	20.90	107.4
		10.00	0.421	11.50	10.90	105.5
		5.00	0.608	6.42	5.90	108.8
2	8.37	0.00	0.518	8.37		
		40.00	0.160	43.57	48.37	90.1
		20.00	0.225	27.59	28.37	97.3
		10.00	0.321	17.00	18.37	92.5
		5.00	0.367	14.07	13.37	105.2
3	14.60	0.00	0.357	14.61		
		40.00	0.144	50.31	54.61	92.1
		20.00	0.187	35.55	34.60	102.7
		10.00	0.246	24.52	24.60	99.7
		5.00	0.279	20.60	19.60	105.1

Linearity

Three samples (saliva) containing different amounts of analyte were serially diluted to 1:64 with zero standard and assayed with the DRG ELISA. The percentage recovery was calculated by comparing the expected and measured values for SLV cortisol. An assay linearity of 0.537 – 77 ng/mL has been identified as the usable range. Samples above this range must be diluted and re-run.

	Sample 1	Sample 2	Sample 3
Concentr. ng/mL	33.13	80.00	23.23
Average % Recovery	107.0	99.1	97.5
Range of from	101.1	97.8	92.4
% Recovery to	114.0	99.6	104.4

Comparison Studies

Studies were performed to compare the DRG SLV Cortisol test to commercially available tests.

One study evaluated saliva samples from 114 subjects ages 40 to 70 years. The samples were run in duplicate on the DRG test and another commercially available LIA method to determine the concentration of Cortisol in the samples. A correlation of 0.872 was obtained versus this method.

A second study was performed using saliva samples from seventy-two (72) saliva samples collected from 40 – 70 year old men and women and run in duplicate on DRG and another commercially available EIA test.

Another study was performed comparing 28 saliva samples to a reference LC-MS method.
A correlation of r = 0.89056 with a formula of y = 1.0144x + 1.7762 was obtained to this method.

To further demonstrate substantial equivalence of the DRG SLV test, additional expanded comparison studies were requested.

One expanded study evaluated saliva samples from 40 subjects ages 25 – 65 years. The samples were run in duplicate on the DRG test and another commercially available LIA method to determine the concentration of Cortisol in the samples. An overall correlation of 0.9795 and a regression formula of y = 0.9588x - 0.0338 was obtained versus this method.

A second expanded study was performed using 40 saliva samples collected from men and women ages 25 – 65 years and run in duplicate on DRG and another commercially available EIA test.
A correlation of 0.9920 with a regression formula of y = 1.0722x + 0.1482 was observed compared to another EIA method.

LIMITATIONS OF USE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice.
Any improper handling of samples or modification of this test might influence the results.

The patient should not eat, drink, chew gum or brush teeth for 30 minutes before sampling. Otherwise rinse mouth thoroughly with cold water 5 min prior to sample collection. Do not collect samples when oral diseases, inflammation or lesions exist (blood contamination).

Interfering Substances

Blood contamination of more than 0.32% in saliva samples will affect results, and usually can be seen by eye.

Concentrations of Sodium Azide >0.2% interferes in this assay and may lead to false results.

High-Dose-Hook Effect

No hook effect was observed in this test.

Legal Aspects

Only for countries where the declaration of European Conformity (CE mark) is applicable.

Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.
The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact DRG.

Therapeutic Consequences

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 11.1. Any laboratory result is only a part of the total clinical picture of a patient.
Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.
The test result itself should never be the sole determinant for deriving any therapeutic consequences.

Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results subject to point 11.2. are also invalid.
Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

Revised 7 April 2014 rm (Vers. 9.1)

REFERENCES

1. Irwin M, et al (1987): Life events, depressive symptoms and immune function, Am J. Psychiat, 144, 437-441
2. Solomon GF, Moss RH. (1964): Emotions, Immunity and disease. A speculative theoretical integration, Arch. Gen Psychiatry, 11, 657-674
3. Mcgrady A. et al (1987): Effect of biofeedback-assisted relaxation in blood pressure and cortisol levels in normotensives and hypertensives, J. Behav. Med., 10, 301-310
4. Hucklebridge FH, et al. (1999): The awakening of cortisol response and blood glucose levels, Life Sci., 64, 931-937
5. Drucker S. (1987): New MI: Disorders of adrenal steroidogenesis, Pediatr. Clin. North Am, 34, 1055-1066
6. Hellhammer DH, et al. (1997): Social hierarchy and adrenocortical stress Reactivity in men, Psychoneuroendocrinology, 22, 643-650
7. Van cauter E. (1987): Pulsatile ACTH secretion . In: Wagner T., Filicori M. (eds): Episodic hormone secretion: From basic science to clinical application, Hameln, TM-Verlag, pp 65-75
8. Chernow B., et al (1987): Hormonal responses to graded surgical stress, Arch. Intern. Med., 147, 1273- 1278
9. Hellhammer DH, et al (1987): Measurement of salivary cortisol under psychological Stimulation, In: Hingten JN, Hellhammer DH, Huppmann (eds.), Advanced methods in Psychology, Hogrefe, Toronto, pp 281-289
10. Riad-Fahny et al (1982), Steroids in saliva for assessing endocrine function, Endocr. Rev, 3, 367-395
11. Kirchbaum C., Hellhammer DH. (1989): Salivary cortisol in psychobiological Research: An overview, Neuropsychobiology, 22, 150-169
12. Kirchbaum C, Hellhammer Dh. (1994): Salivary cortisol in psychoneuroendocrine Research: Recent developments and applications, Psychoneuroendocrinology, 19, pp 313-333
13. Robin P., et al. (1977): Assay of unbound cortisol in plasma, J. Clin. Endocrinol. Metab., 46, 277-283
14. Vining RF, et al. (1983), Hormones in saliva: Mode of entry and consequent implications for clinical interpretation, Clin. Chem., 29, 1752-1756

Rev. 2014-04-03cc