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EXPANDED RANGE
High Sensitivity
SALIVARY CORTISOL ENZYME IMMUNOASSAY KIT

Catalog No. 1-3102/1-3112, 96-Well Kit

Intended Use

The Salimetrics™ cortisol kit is a competitive immunoassay specifically designed and validated for the quantitative *in vitro* diagnostic measurement of salivary cortisol. This kit may be used to measure adrenal cortical function and as a screen for Cushing's and Addison's disease (1,2). Saliva cortisol accurately reflects the amount of serum cortisol in the circulation. Salimetrics has not validated this kit for use with serum or plasma samples. Please read the complete kit insert before performing this assay. For further information about this kit, its application, or the procedures in this insert, please contact the technical service team at Salimetrics or your local sales representative.

Introduction

Cortisol (hydrocortisone, Compound F) is the major glucocorticoid produced in the adrenal cortex. Cortisol is actively involved in the regulation of calcium absorption, blood pressure maintenance, anti-inflammatory function, gluconeogenesis, gastric acid and pepsin secretion, and immune function (3,4,5).

Cortisol production has a circadian rhythm (6). Levels peak in the early morning and drop to the lowest concentration at night (7). Levels rise independently of circadian rhythm in response to stress (8). Increased cortisol production is associated with Cushing's syndrome and adrenal tumors, while decreased cortisol production is associated with adrenal insufficiency (e.g., Addison's disease) and adrenocorticotrophic hormone (ACTH) deficiency (9).

In the blood only 1 to 15% of cortisol is in its unbound or biologically active form. The remaining cortisol is bound to serum proteins (10). Unbound serum cortisol enters the saliva via intracellular mechanisms, and in saliva the majority of cortisol remains unbound to protein (11). Salivary cortisol levels are unaffected by salivary flow rate or salivary enzymes (12). Studies consistently report high correlations between serum and saliva cortisol, indicating that salivary cortisol levels reliably estimate serum cortisol levels (13,14,15).

Test Principle

A microtitre plate is coated with monoclonal antibodies to cortisol. Cortisol in standards and unknowns competes with cortisol linked to horseradish peroxidase for the antibody binding sites. After incubation, unbound components are washed away. Bound cortisol peroxidase is measured by the reaction of the peroxidase enzyme on the substrate tetramethylbenzidine (TMB). This reaction produces a blue color. A yellow color is formed after stopping the reaction with sulfuric acid. Optical density is read on a standard plate reader at 450 nm. The amount of cortisol peroxidase detected, as measured by the intensity of color, is inversely proportional to the amount of cortisol present (16).

pH Indicator

A pH indicator in the assay diluent alerts the user to samples with high or low pH values. Acidic samples will turn the diluent yellow. Alkaline samples will turn the diluent purple. Dark yellow or purple wells indicate that a pH value for that sample should be obtained using pH strips. Cortisol values from samples with a pH ≤ 3.5 or ≥ 9.0 may be artificially inflated or lowered (17).

Precautions

1. Liquid stop solution is a 3-molar solution of sulfuric acid. This solution is caustic; use with care. Stop solution in powdered form is not sulfuric acid-based and is mildly corrosive.
2. This kit uses break-apart microtitre strips. Unused wells must be stored at 2 - 8°C in the sealed foil pouch with desiccant and used in the frame provided.
3. Do not mix components from different lots of kits.

4. When using a multichannel pipette, reagents should be added to duplicate wells at the same time. Follow the same sequence when adding additional reagents so that incubation time with reagents is the same for all wells.
5. See 'Material Safety Data' at the end of procedure.
6. We recommend that samples be screened for possible blood contamination (18,19) using a reliable screening tool such as the Salimetrics Blood Contamination EIA Kit (Cat. No.: 1-1302/1-1312). Do not use dipsticks, which result in false positive values due to salivary enzymes.
7. Routine calibration of pipettes is critical for the best possible assay performance.
8. Pipetting of samples and reagents must be done as quickly as possible (without interruption) across the plate.
9. When running multiple plates, or multiple sets of strips, a standard curve should be run with each individual plate and/or set of strips.
10. The temperature of the laboratory may affect assays. Salimetrics' kits have been validated at 68 - 74°F (20 - 23.3°C). Higher or lower temperatures will cause an increase or decrease in OD values, respectively. Salimetrics cannot guarantee test results outside of this temperature range.
11. The quantity of reagent provided with this kit is sufficient for three individual runs. The volume of diluent and conjugate used for assays using less than a full plate should be scaled down accordingly, keeping the same dilution ratio.
12. Avoid microbial contamination of opened reagents. Salimetrics recommends using opened reagents within one month.

Storage

All components of this kit are stable at 2 - 8°C until the kit's expiration date.

Reagents and Reagent Preparation

1. **Anti-Cortisol Coated Plate:** A ready-to-use, 96-well microtitre plate pre-coated with monoclonal anti-cortisol antibodies in a resealable foil pouch.
2. **Cortisol Standard:** Six vials, 500 μ L each, labeled A-F, containing cortisol concentrations of 3.0, 1.0, 0.333, 0.111, 0.037, and 0.012 μ g/dL, in a synthetic saliva matrix with a non-mercury preservative. (Values in nmol/L are 82.77, 27.59, 9.19, 3.06, 1.02, and 0.33 nmol/L respectively.) Standards are traceable to the NIST standard.
3. **Cortisol Controls:** Two controls representing high and low levels of salivary cortisol in a saliva-like matrix with a non-mercury preservative. Each vial contains 0.5 mL. See vials for target values.
4. **Wash Buffer:** 100 mL of a 10X phosphate buffered solution containing detergents and a non-mercury preservative. Dilute only the amount needed for current day's use. Discard any leftover reagent. Dilute the wash buffer concentrate 10-fold with room temperature deionized water (100 mL of 10X wash buffer to 900 mL of deionized H₂O). (**Note:** *If precipitate has formed in the concentrated wash buffer, it may be heated to 60°C for 15 minutes. Cool to room temperature before use in assay.*)
5. **Assay Diluent:** 63 mL of a phosphate buffered solution containing a pH indicator and a non-mercury preservative.
6. **Enzyme Conjugate:** 50 μ L of a solution of cortisol labeled with horseradish peroxidase. Dilute prior to use with assay diluent.
7. **Tetramethylbenzidine (TMB):** 25 mL of a non-toxic, ready-to-use solution.
8. **Stop Solution:** 12.5 mL of a solution of sulfuric acid. Stop solution is provided in powdered form to some customers outside the USA. Reconstitute the powdered stop solution with 12.5 mL of deionized water. Let sit for 10 minutes before use.
9. **Non-specific Binding Wells (NSB):** These wells do not contain anti-cortisol antibody. In order to support multiple use, a strip of NSB wells is included. They are located in the foil pouch. Wells may be broken off and inserted where needed.

Materials Needed But Not Supplied

- Precision pipette to deliver 15 and 25 μ L
- Precision multichannel pipette to deliver 50 μ L and 200 μ L
- Vortex
- Plate rotator (if unavailable, tap plate to mix)
- Plate reader with a 450 nm filter
- Log-linear graph paper or computer software for data reduction
- Deionized water
- Reagent reservoirs
- One disposable tube capable of holding 24 mL
- Pipette tips
- Serological pipette to deliver up to 24 mL

Specimen Collection

Donors may collect whole saliva by tilting the head forward, allowing the saliva to pool on the floor of the mouth, then passing the saliva through a short straw into a polypropylene vial. Adult samples and samples from children ages 6 and above may also be collected using the Salimetrics Oral Swab (SOS), P/N 5001.02. Infant

samples may be collected with the Sorbette, P/N 5029, or cotton ropes, P/N 5016.00. Collection protocols are available on request. For accurate results Sorbettes and cotton collection materials should be completely saturated before removal. Do **not** add sodium azide to saliva samples as a preservative. Samples visibly contaminated with blood should be recollected.

Avoid sample collection within 60 minutes after eating a major meal or within 12 hours after consuming alcohol. Bovine hormones normally present in dairy products can cross-react with anti-cortisol antibodies and cause false results. Acidic or high sugar foods can compromise assay performance by lowering sample pH and influencing bacterial growth. To minimize these factors, rinse mouth thoroughly with water 10 minutes before sample is collected. It is important to record the time and date of specimen collection when samples are obtained due to the diurnal variation in cortisol levels. Samples for Cushing's diagnosis should be collected at 11:00 pm. After collection it is important to keep samples cold, in order to avoid bacterial growth in the specimen. Refrigerate samples within 30 minutes, and freeze at or below -20°C within 4 hours after collection. (Samples may be stored at -20°C or lower for long-term storage.)

Freezing saliva samples will precipitate the mucins. On day of assay, thaw completely, vortex, and centrifuge at 1500 x g (@3000 rpm) for 15 minutes. Avoid multiple freeze-thaw cycles. However, if samples have been refrozen, centrifuge again prior to assaying. Samples should be at room temperature before adding to assay plate. Pipette clear sample into appropriate wells. Particulate matter may interfere with antibody binding, leading to falsely elevated results.

Procedure

Bring all reagents to room temperature. **Note:** It is important to keep the zip-lock pouch with the plate strips closed until warmed to room temperature as humidity may have an effect on the coated wells. Mix all reagents before use.

Step 1: Determine your plate layout. Here is a suggested layout.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|------------|------------|-------|-------|---|---|---|---|---|----|----|----|
| A | 3.000 Std | 3.000 Std | C-H | C-H | | | | | | | | |
| B | 1.000 Std. | 1.000 Std. | C-L | C-L | | | | | | | | |
| C | 0.333 Std | 0.333 Std | Unk-1 | Unk-1 | | | | | | | | |
| D | 0.111 Std | 0.111 Std | Unk-2 | Unk-2 | | | | | | | | |
| E | 0.037 Std | 0.037 Std | Unk-3 | Unk-3 | | | | | | | | |
| F | 0.012 Std | 0.012 Std | Unk-4 | Unk-4 | | | | | | | | |
| G | Zero | Zero | Unk-5 | Unk-5 | | | | | | | | |
| H | NSB | NSB | Unk-6 | Unk-6 | | | | | | | | |

Step 2: Keep the desired number of strips in the strip holder and place the remaining strips back in the foil pouch. If you choose to place non-specific binding wells in H-1, 2, remove strips 1 and 2 from the strip holder and break off the bottom wells. Place the strips back into the strip holder leaving H-1, 2 blank. Break off 2 NSB wells from the strip of NSBs included in the foil pouch. Place in H-1, 2. Alternatively, NSBs may be placed wherever you choose on the plate. Reseal the zip-lock foil pouch with unused wells and desiccant. Store at 2 - 8°C.

Caution: Extra NSB should not be used for determination of standards, controls or unknowns.

Step 3:

- Pipette 24 mL of assay diluent into a disposable tube. Set aside for Step 5.

Step 4:

- Pipette 25 µL of standards, controls, and unknowns into appropriate wells. Standards, controls, and unknowns should be assayed in duplicate.
- Pipette 25 µL of assay diluent into 2 wells to serve as the zero value.
- Pipette 25 µL of assay diluent into each NSB well.

Step 5: Make a 1:1600 dilution of the conjugate by adding 15 µL of the conjugate to the 24 mL of assay diluent prepared in Step 3. (Scale down proportionally if not using the entire plate.) Immediately mix the diluted conjugate solution and pipette 200 µL into each well using a multichannel pipette.

Step 6: Mix plate on rotator for 5 minutes at 500 rpm (or tap to mix) and incubate at room temperature for an additional 55 minutes.

Step 7: Wash the plate 4 times with 1X wash buffer. A plate washer is recommended. However, washing may be done by gently squirting wash buffer into each well with a squirt bottle, or by pipetting 300 µL of wash buffer into each well, and then discarding the liquid by inverting the plate over a sink. After each wash, the plate should be thoroughly blotted on paper towels before being turned upright. *If using a plate washer, blotting is still recommended after the last wash.*

Step 8: Add 200 µL of TMB solution to each well with a multichannel pipette.

Step 9: Mix on a plate rotator for 5 minutes at 500 rpm (or tap to mix) and incubate the plate in the dark at room temperature for an additional 25 minutes.

Step 10: Add 50 µL of stop solution with a multichannel pipette.

Step 11:

- Mix on a plate rotator for 3 minutes at 500 rpm (or tap to mix). **Caution:** *Do not mix at speeds over 600 rpm.*
- Wipe off bottom of plate with a water-moistened, lint-free cloth and wipe dry.
- Read in a plate reader at 450 nm. Read plate within 10 minutes of adding stop solution. (Correction at 490 to 630 is desirable.)

Calculations

- Compute the average optical density (OD) for all duplicate wells.
- Subtract the average OD for the NSB wells from the average OD of the zero, standards, controls, and unknowns.
- Calculate the percent bound (B/Bo) for each standard, control, and unknown by dividing the average OD (B) by the average OD for the zero (Bo).
- Determine the concentrations of the controls and unknowns by interpolation using software capable of logistics. We recommend using a 4-parameter sigmoid minus curve fit.

Limitations

- Samples with cortisol values greater than 3.0 µg/dL (82.77 nmol/L) should be diluted with assay diluent and rerun for accurate results. To obtain the final cortisol concentration, multiply the concentration of the diluted sample by the dilution factor.
- Salimetrics has not validated this kit to measure cortisol levels in serum or plasma.
- Diagnosis of Cushing's syndrome should be confirmed by additional diagnostic tests for the disease, such as low-dose dexamethasone suppression testing.
- A pH value should be obtained on samples that appear yellow or purple after assay diluent is added and the plate is mixed. Samples with pH values ≥ 9 or ≤ 3.5 should be recollected.
- Saliva samples contaminated with blood may give false results (18,19). Samples may be screened for blood contamination using a reliable screening tool such as the Salimetrics Blood Contamination EIA Kit (Cat. No.: 1-1302 or 1-1312).
- Cortisol levels are elevated during the later stages of pregnancy and in women on oral contraceptives or after long-term use of oral contraceptives (15, 20).
- Some studies show developmental differences in cortisol as well as an association between cortisol and weight (21).
- Elevated cortisol levels can be found in conditions of sepsis, infection, chronic liver disease, and renal failure. Low cortisol levels result from liver disease, pituitary hyposecretion, hypothyroidism, or steroid therapy.
- See "Specimen Collection" recommendations to insure proper collection of saliva specimens and to avoid interfering substances.
- Freeze all saliva samples prior to assay in order to precipitate mucins and facilitate pipetting.
- Saliva samples must be centrifuged before assay. Particulate matter may interfere with antibody binding, leading to falsely elevated results.
- Samples collected with sodium azide are unsuitable for this assay.

Quality Control

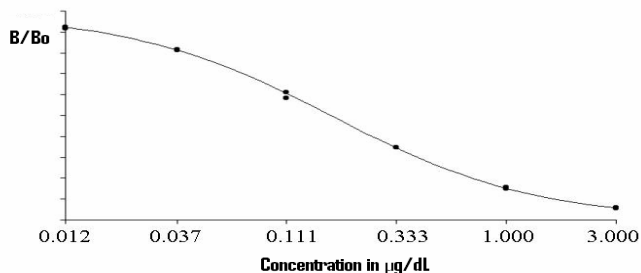
The Salimetrics' high and low salivary cortisol controls should be run with each assay. The control ranges established at Salimetrics are to be used as a guide. Each laboratory should establish its own range. Variations between laboratories may be caused by differences in techniques and instrumentation.

Typical Results

The following charts and graphs are for illustration only and should not be used to calculate results from another assay.

| Well | Sample | Average OD | B | B/Bo | Cortisol (µg/dL) |
|-------|--------|------------|-------|-------|------------------|
| A1,A2 | S1 | 0.094 | 0.071 | 0.048 | 3.000 |
| B1,B2 | S2 | 0.236 | 0.213 | 0.145 | 1.000 |
| C1,C2 | S3 | 0.524 | 0.501 | 0.340 | 0.333 |
| D1,D2 | S4 | 0.897 | 0.874 | 0.593 | 0.111 |
| E1,E2 | S5 | 1.219 | 1.196 | 0.812 | 0.037 |
| F1,F2 | S6 | 1.379 | 1.356 | 0.921 | 0.012 |
| G1,G2 | Bo | 1.496 | 1.473 | NA | NA |
| H1,H2 | NSB | 0.023 | NA | NA | NA |

Example: Cortisol 4-Parameter Sigmoid Minus Curve Fit



Material Safety Data*

Hazardous Ingredients

Liquid stop solution is caustic; use with care. *Note: Stop solution in powdered form is not sulfuric acid-based and is mildly corrosive.*

We recommend the procedures listed below for all kit reagents. Specific kit component MSDS sheets are available from Salimetrics upon request.

Handling

Follow good laboratory procedures when handling kit reagents. Laboratory coats, gloves, and safety goggles are recommended. Wipe up spills using standard absorbent materials while wearing protective clothing. Follow local regulations for disposal.

Emergency Exposure Measures

In case of contact, immediately wash skin or flush eyes with water for 15 minutes. Remove contaminated clothing. If inhaled, remove individual to fresh air. If individual experiences difficulty breathing, give oxygen and call a physician.

*The above information is believed to be accurate but is not all-inclusive. This information should be used only as a guide. Salimetrics will not be liable for accidents or damage resulting from contact with reagents.

HS Cortisol EIA Assay Performance Characteristics

Linearity of Dilution: Two saliva samples were diluted with assay diluent and assayed.

| Sample | Dilution Factor | Expected (µg/dL) | Observed (µg/dL) | Recovery (%) |
|--------|-----------------|------------------|------------------|--------------|
| 1 | | | 2.176 | |
| | 1:2 | 1.088 | 1.065 | 97.9 |
| | 1:4 | 0.544 | 0.503 | 92.5 |
| | 1:8 | 0.272 | 0.233 | 85.7 |
| | 1:16 | 0.136 | 0.109 | 80.1 |
| 2 | | | 0.508 | |
| | 1:2 | 0.254 | 0.247 | 97.2 |
| | 1:4 | 0.127 | 0.118 | 92.9 |
| | 1:8 | 0.064 | 0.058 | 90.6 |
| | 1:16 | 0.032 | 0.031 | 96.9 |

Recovery: Six saliva samples containing different levels of endogenous cortisol were spiked with known quantities of cortisol and assayed.

| Sample | Endogenous (µg/dL) | Added (µg/dL) | Expected (µg/dL) | Observed (µg/dL) | Recovery (%) |
|--------|--------------------|---------------|------------------|------------------|--------------|
| 1 | 0.088 | 2.000 | 2.088 | 2.176 | 104.2 |
| 2 | 0.077 | 0.300 | 0.377 | 0.380 | 100.8 |
| 3 | 0.062 | 0.011 | 0.073 | 0.071 | 97.3 |
| 4 | 0.066 | 2.500 | 2.566 | 2.723 | 106.1 |
| 5 | 0.210 | 0.300 | 0.510 | 0.508 | 99.6 |
| 6 | 0.086 | 0.011 | 0.097 | 0.094 | 96.9 |

Precision:

1. The intra-assay precision was determined from the mean of 14 (low) and 18 (high) replicates each.

| Sample | N | Mean (µg/dL) | Standard Deviation (µg/dL) | Coefficient of Variation (%) |
|---------|----|--------------|----------------------------|------------------------------|
| Level 1 | 18 | 0.999 | 0.033 | 3.35 |
| Level 2 | 14 | 0.097 | 0.004 | 3.65 |

2. The inter-assay precision was determined from the mean of average duplicates for 12 separate runs.

| Sample | N | Mean (µg/dL) | Standard Deviation (µg/dL) | Coefficient of Variation (%) |
|---------|----|--------------|----------------------------|------------------------------|
| Level 1 | 12 | 1.020 | 0.038 | 3.75 |
| Level 2 | 12 | 0.101 | 0.006 | 6.41 |

Sensitivity: The lower limit of sensitivity was determined by interpolating the mean minus 2 SDs for 10 sets of duplicates at 0 µg/dL standard. The minimal concentration of cortisol that can be distinguished from 0 is < 0.003 µg/dL.

Correlation with Serum: The correlation between serum and saliva cortisol was determined by assaying 49 matched samples using the Diagnostic Systems Laboratories' serum Cortisol EIA and the Salimetrics ER HS Salivary Cortisol EIA.

The correlation between saliva and serum was highly significant, $r(47) = 0.91$, $p < 0.0001$.

Specificity of Antiserum

| Compound | Spiked Concentration (ng/mL) | % Cross-reactivity in ER HS Salivary Cortisol EIA |
|----------------------------------|------------------------------|---|
| Prednisolone | 100 | 0.568 |
| Prednisone | 1000 | ND |
| Cortisone | 1000 | 0.130 |
| 11-Deoxycortisol | 500 | 0.156 |
| 21-Deoxycortisol | 1000 | 0.041 |
| 17 α -Hydroxyprogesterone | 1000 | ND |
| Dexamethasone | 1000 | 19.2 |
| Triamcinolone | 1000 | 0.086 |
| Corticosterone | 10,000 | 0.214 |
| Progesterone | 1000 | 0.015 |
| 17 β -Estradiol | 10 | ND |
| DHEA | 10,000 | ND |
| Testosterone | 10,000 | 0.006 |
| Transferrin | 66,000 | ND |
| Aldosterone | 10,000 | ND |

ND = None detected (<0.004)

Salivary Cortisol Expected Ranges

Each laboratory should establish its own range of expected values. The following values have been reported for salivary cortisol.

| Group | Number | Overall Range in µg/dL |
|------------------------|--------|------------------------|
| Children, neonatal | 275 | ND - 3.417 |
| Children, age 6 months | 165 | ND - 2.734 |

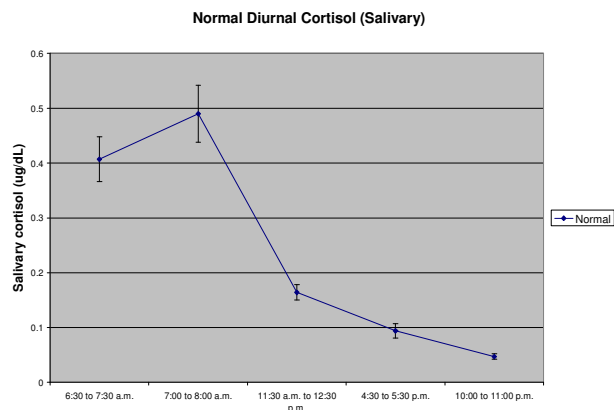
| Group | Number | AM Range in µg/dL | PM Range in µg/dL |
|---------------------------|--------|-------------------|-------------------|
| Children, ages 2.5-5.5 | 112 | 0.034 - 0.645 | 0.053 - 0.607 |
| Children, ages 8-11 | 285 | 0.084 - 0.839 | ND - 0.215 |
| Adolescents, ages 12-18 | 403 | 0.021 - 0.883 | ND - 0.259 |
| Adult males, ages 21-30 | 26 | 0.112 - 0.743 | ND - 0.308 |
| Adult females, ages 21-30 | 20 | 0.272 - 1.348 | ND - 0.359 |
| Adult males, ages 31-50 | 67 | 0.122 - 1.551 | ND - 0.359 |
| Adult females, ages 31-50 | 31 | 0.094 - 1.515 | ND - 0.181 |
| Adult males, ages 51-70 | 28 | 0.112 - 0.812 | ND - 0.228 |
| Adult females, ages 51-70 | 23 | 0.149 - 0.739 | 0.022 - 0.254 |
| All adults | 192 | 0.094 - 1.551 | ND - 0.359 |

| Group | Number | 2300h (ug/dL) |
|--------------------|--------|---------------|
| Normal subjects | 19 | 0.007 - 0.115 |
| Cushing's subjects | 21 | 0.130 - 2.972 |

ND = None detected

Expected ranges for neonates to 5.5 years were derived using the Salimetrics Salivary Cortisol Immunoassay Kit.

Expected ranges for 8 to 18 years were reported from an unpublished manuscript, Pennsylvania State University's Behavioral Endocrinology Laboratory. Adult ranges were obtained from published literature (22).



Note: Time of cortisol peak will vary in individual patients relative to their normal wake-up time.

References

- Raff, H., Raff, J.L., & Findling, J.W. (1998). Late-night salivary cortisol as a screening test for Cushing's Syndrome. *J Clin Endocrinol Metab*, 83, 2681-2686.
- Papanicolaou, D.A., Mullen, N., Kyrou, I., & Nieman, L.K. (2002). Nighttime salivary cortisol: a useful test for the diagnosis of Cushing's Syndrome. *J Clin Endocrinol Metab*, 87, 4515-21.
- Migeon, C.J., & Lanes, R.L. (1990). Adrenal cortex: hypo- and hyperfunction. In F. Lifshitz (ed.), *Pediatric endocrinology, a clinical guide* (2nd ed.), (pp. 333-352). New York: Marcel Dekker.
- Drucker, S. (1987). New MI: Disorders of adrenal steroidogenesis. *Pediatr Clin North Am*, 34, 1055-1066.
- Fischbach, F.T. (1992). *The manual of laboratory and diagnostic tests*, (4th ed.). Philadelphia: J. B. Lippincott.
- Dorn, L.D., Lucke, J. F., Loucks, T.L., Berga, S.L. (2007). Salivary cortisol reflects serum cortisol: analysis of circadian profiles. *Ann Clin Biochem*, 44, 281-284.
- Chernow, B., et al. (1987). Hormonal responses to graded surgical stress. *Arch Intern Med*, 147, 1273-1278.
- Kreiger, D.T. (1975). Rhythms of ACTH and corticosteroid secretion in health and disease and their experimental modification. *J Steroid Biochem*, 6, 758-791.
- Rothfield, B. (1974). Plasma cortisol. In: B. Rothfield (Ed.), *Nuclear medicine-in vitro* (pp. 120-5). Philadelphia: Lippincott.
- Robin, P., Predine, J., & Milgrom, E. (1977). Assay of unbound cortisol in plasma. *J Clin Endocrinol Metab*, 46, 277-283.
- Vining, R.F., McGinley, R.A., & Symons, R.G. (1983). Hormones in saliva: mode of entry and consequent implications for clinical interpretation. *Clin Chem*, 29, 1752-1756.
- Vining, R.F., & McGinley, R.A. (1987). The measurement of hormones in saliva: Possibilities and pitfalls. *J Steroid Biochem*, 27, 81-94.
- Francis, S.J., Walker, R.F., Riad-Fahmy, D., Hughes, D., Murphy, J.F., Gray, O.P. (1987). Assessment of adrenocortical activity in term newborn infants using salivary cortisol determinations. *J of Pediatrics*, 111, 129-133.
- Hiramatsu, R. (1981). Direct assay of cortisol in human saliva by solid phase radioimmunoassay and its clinical applications. *Clinica Chimica Acta*, 117, 239-249.
- Vining, R.F., McGinley, R.A., Maksvytis, J.J., & Ho, K.Y. (1983). Salivary cortisol: A better measure of adrenal cortical function than serum cortisol. *Annals Clin Biochem*, 20, 329-335.
- Chard, T. (1990). *An introduction to radioimmunoassay and related techniques*. Amsterdam: Elsevier.
- Schwartz, E.B., Granger, D.A., Susman, E.J., Gunnar, M.R., & Laird, B. (1998). Assessing salivary cortisol in studies of child development. *Child Development*, 69, 1503-1513.
- Kivlighan, K.T., Granger, D.A., Schwartz, E.B., Nelson, V., & Curran, M. (2004). Quantifying blood leakage into the oral mucosa and its effects on the measurement of cortisol, dehydroepiandrosterone, and testosterone in saliva. *Hormones and Behavior*, 46, 39-46.

- Schwartz, E., & Granger, D.A. (2004). Transferrin enzyme immunoassay for quantitative monitoring of blood contamination in saliva. *Clinical Chemistry*, 50, 654-656.
- Susman, E.J., Schmeelk, K.H., Worrall, B.S., Granger, D.A., Ponirakis, A., & Chrousos, M.D. (1999). Corticotropin-releasing hormone and cortisol: Longitudinal associations with depression and antisocial behavior in pregnant adolescents. *Journal of the American Academy of Child and Adolescent Psychiatry*, 38, 460-467.
- Kiess, W., Meidert, A., Dressendorfer, R.A., Schriever, K., Kessler, U., Konig, A., Schwarz, H.P., & Strasburger, C.J. (1995). Salivary cortisol levels throughout childhood and adolescence: Relation with age, pubertal state, and weight. *Pediatric Research*, 37, 502-506.
- Aardal, E., Holm, A. (1995). Cortisol in saliva-reference ranges in relation to cortisol in serum. *Eur J Clin Chem Clin Biochem*, 33, 927-932.

Seller's Limited Warranty

"Seller warrants that all goods sold hereunder will be free from defects in material and workmanship. Upon prompt notice by Buyer of any claimed defect, which notice must be sent within thirty (30) days from date such defect is first discovered and within three months from the date of shipment, Seller shall, at its option, either repair or replace the product that is proved to Seller's satisfaction to be defective. All claims should be submitted in writing. This warranty does not cover any damage due to accident, misuse, negligence, or abnormal use. Liability in all cases, will be limited to the purchased cost of the kit.

It is expressly agreed that this limited warranty shall be in lieu of all warranties of fitness and in lieu of the warranty of merchantability. Seller shall not be liable for any incidental or consequential damages that arise out of the installation, use or operation of Seller's product or out of the breach of any express or implied warranties."

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