GENOVA DIAGNOSTICS
63 Zillicoa Street
Asheville, NC 28801

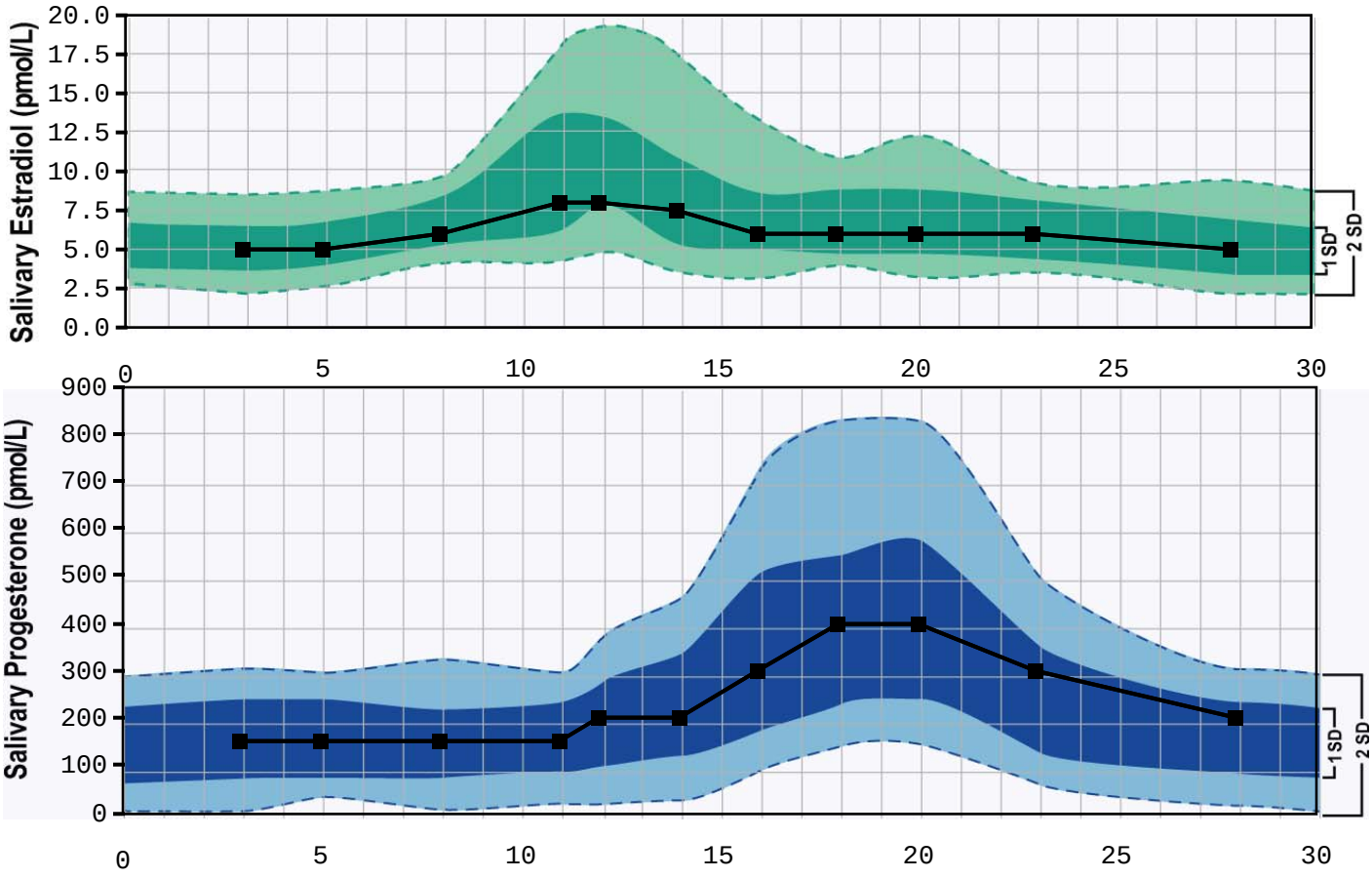
Rhythm Plus™

Patient: **SAMPLE PATIENT**
DOB:
Sex:
MRN:

4102 Rhythm Plus - Saliva

Methodology: EIA, LIA

Estradiol & Progesterone Activity plus Testosterone Level



Day of Cycle	3	5	8	11	12	14	16	18	20	23	28	Avg.
Estradiol	5.0	5.0	6.0	8.0	8.0	7.5	6.0	6.0	6.0	6.0	5.0	6.3
Progesterone	150	150	150	150	200	200	300	400	400	300	200	236
P/E2 Ratio	30	30	25	19	25	27	50	67	67	50	40	39

Estradiol	Ref Range
Follicular:	2.8 - 8.8 pmol/L
Peak*:	4.5 - 19.1 pmol/L
Luteal:	2.8 - 8.2 pmol/L
Menopausal:	3.7 - 9.4 pmol/L
Male:	3.1 - 7.4 pmol/L

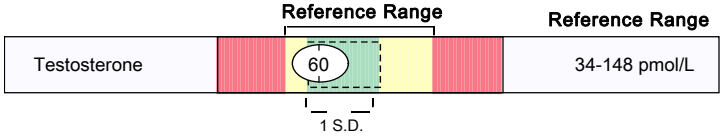
* Peak = Days 11 and 12

Progesterone	Ref Range
Follicular:	17 - 321 pmol/L
Peak*:	151 - 829 pmol/L
Luteal:	33 - 452 pmol/L
Menopausal:	45 - 370 pmol/L
Male:	31 - 280 pmol/L

* Peak = Days 18 and 20

P/E2 Ratio ♦	Ref Range
Follicular:	10 - 85
Luteal:	8 - 80
Menopausal:	12 - 62

Testosterone	Ref Range
Premenopausal:	34 - 148 pmol/L
Menopausal:	34 - 148 pmol/L
Male:	110 - 513 pmol/L



Commentary

The performance characteristics of all assays have been verified by Genova Diagnostics, Inc.
All assays are cleared by the U.S. Food and Drug Administration unless otherwise noted with ♦.

Commentary is provided to the practitioner for educational purposes, and should not be interpreted as diagnostic or as treatment recommendations. Diagnosis and treatment decisions are the practitioner's responsibility.

Reference ranges are based on morning collection.

The Reference Range for each day is a statistical interval representing 95% or 2 Standard Deviations (2 S.D.) of the reference population. One Standard Deviation (1 S.D.) is a statistical interval representing 68% of the reference population. Values between 1 and 2 S.D. are not necessarily abnormal. Clinical correlation is suggested.

The first half of the menstrual cycle (Follicular Phase) culminates in an estradiol peak between days 10-14 (in an optimal 28-day cycle – counting from the first day of the last menses). The second half of a 28-day menstrual cycle (Luteal Phase) should demonstrate a progesterone peak between days 18-22, which coincides with a smaller estradiol rise. Ovulation occurs 24-36 hours after the estradiol peak and 10-12 hours after a luteinizing hormone (LH) surge. Alterations in this normal hormonal cycling may be indicative of anovulation or luteal phase defects, which are associated with menstrual bleeding abnormalities. Finally, menstrual cycle lengths often vary from 24-35 days. While the follicular phase may vary in duration, the luteal phase is fixed at 14 days.

Follicular estradiol:
High follicular estradiol levels contribute to menstrual irregularities, breast tenderness, and estrogen-related conditions such as ovarian cysts, endometrial hyperplasia, and uterine fibroids. Low follicular estradiol levels can occur with normal aging, ovarian dysfunction, low body mass, strenuous exercise, chronic stress, or oral contraceptive use.

Follicular progesterone:
Elevated follicular progesterone levels represent HPA axis activity or a persistent corpus luteum from the previous cycle. This finding is not necessarily associated with symptoms, but may accompany prolonged bleeding or polycystic ovary syndrome.
Low follicular progesterone levels are seen in ovarian aging.

Luteal estradiol:
Elevated luteal estradiol levels are seen in decreased estrogen detoxification, high body mass index, hypothyroidism, or transdermal estradiol supplementation. High luteal estradiol contributes to disorders such as PMS, dysmenorrhea, and dysfunctional uterine bleeding. Low luteal estradiol levels on one or more days may result from ovarian insufficiency, low body mass, strenuous exercise, chronic stress, inflammation, or certain medications, including oral contraceptives. Low luteal estradiol is associated with anovulation, scanty periods, or depression-type PMS.

Luteal progesterone:
High luteal progesterone levels are present in some types of PMS, particularly those associated with fatigue, depression and blood sugar dysregulation. Elevated progesterone can also reflect recent transdermal progesterone supplementation.
Low luteal progesterone occurs with luteal defects, anovulation, chronic stress, and certain medications including oral contraceptives. Deficient luteal progesterone is a leading cause of infertility and dysfunctional uterine bleeding, and is relatively common as a woman approaches menopause.
Luteal defects occur when the corpus luteum fails to produce progesterone. In some cases there may be recovery of

Commentary

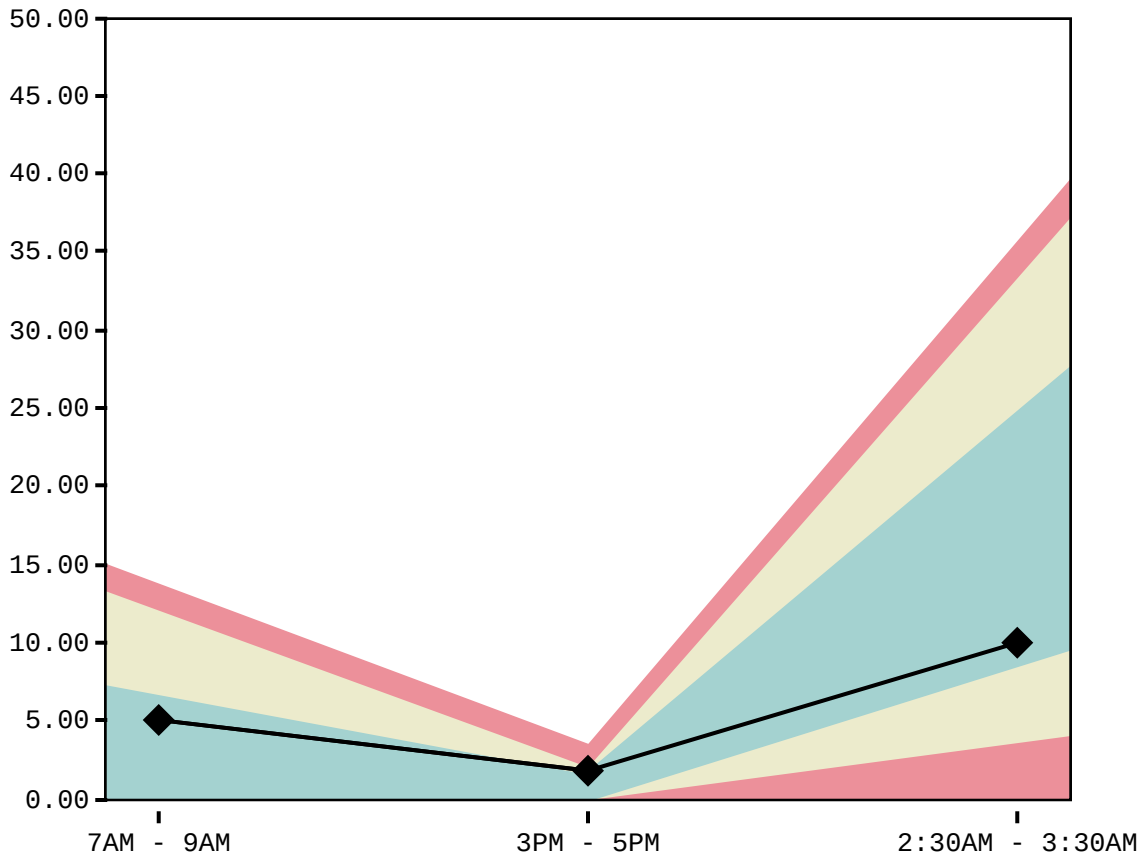
corpus luteal function with a progesterone level rebound. This situation is relatively common as women age, and is a frequent cause of infertility and recurrent miscarriage. It also contributes to dysfunctional uterine bleeding and PMS.

Testosterone:
Normal testosterone levels are important for libido, maintaining lean body mass and bone density. Low testosterone is associated with greater osteoporosis risk, difficulty maintaining lean body mass, decreased libido, effects of aging, and/or ovarian dysfunction.
High testosterone levels in women are seen with polycystic ovary syndrome, acne, hair loss, glucose intolerance, and ovarian dysfunction.

Comprehensive Melatonin Profile

Methodology: EIA

Salivary Melatonin



Results

	7AM-9AM*	3PM-5PM*	2:30AM - 3:30AM*
Patient Results (pg/mL) >>	**5.00**	**1.80**	**10.00**
Reference Range (pg/mL) *Based on Collection Times	<=12.12	<=1.97	3.71-33.38

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Melatonin activity is normal throughout the sample period revealing a normal melatonin circadian rhythm.

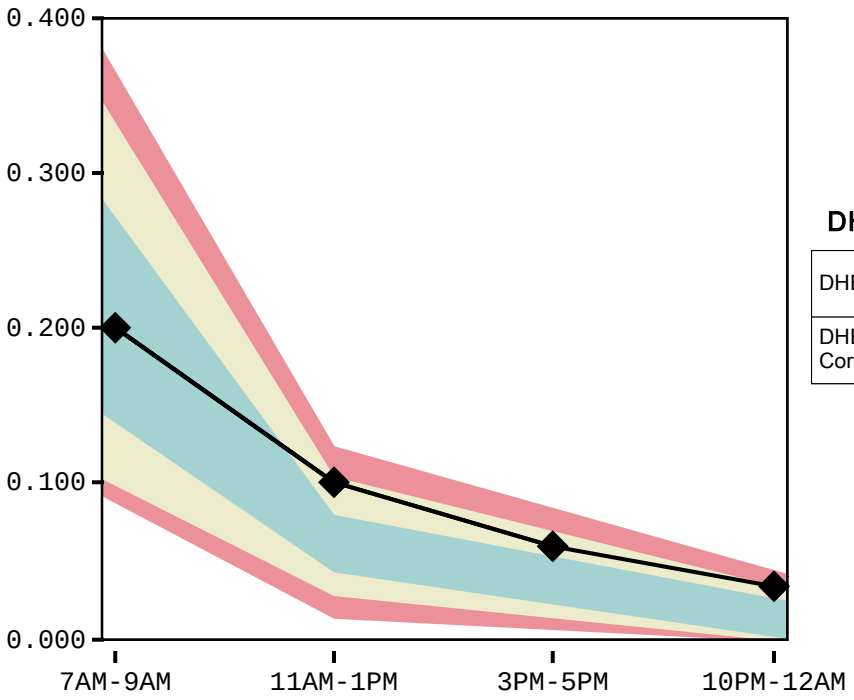
As well as playing a crucial role in sleep-wake cycles, melatonin influences other vital functions, including cardiovascular and antioxidant protection, endocrine function, immune regulation and body temperature.

Adrenocortex Stress Profile

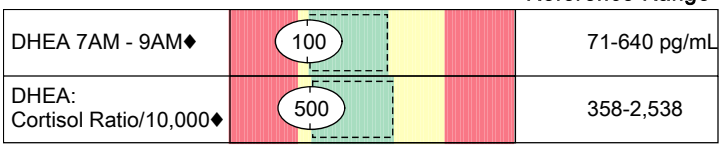
Methodology: EIA

Salivary Cortisol and DHEA

Salivary Cortisol



DHEA



Results

	7AM-9AM*	11AM-1PM*	3PM-5PM*	10PM-12AM*
Patient Result (mcg/dL) >>	**0.200**	**0.100**	**0.060**	**0.034**
Reference Range (mcg/dL) *Based on Collection Times	0.097-0.337	0.027-0.106	0.013-0.068	<=0.034
Actual Collection Time	7:00AM	11:00AM	4:00PM	10:00PM

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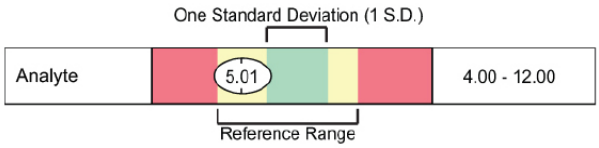
The **Reference Range** is a statistical interval representing 95% or 2 Standard Deviations (2 S.D.) of the reference population.

Commentary

One Standard Deviation (1 S.D.) is a statistical interval representing 68% of the reference population. Values between 1 and 2 S.D. are not necessarily abnormal. Clinical correlation is suggested. (See example below)



Diurnal Cortisol Rhythm/Slope

The natural cortisol diurnal rhythm shows a peak within the first hour after awakening, a rapid decline over the morning hours, and then a tapering through the rest of the day before reaching a nighttime nadir.

A flat slope is characterized by low morning levels, blunted afternoon response and/or evening drop in cortisol levels. Flattened slopes are:

- Associated with a chronic stress burden, poor psychosocial functions, lack of HPA axis resiliency and lower perceived control over stress.
- Predictive of health outcomes, such as increased breast cancer mortality, increased coronary calcifications, and increased body mass index.
- Seen in Post-Traumatic Stress Disorder (PTSD), persistent fatigue, anxiety, depression, and Addison's Disease.

A "high flat" slope is characterized by high morning levels that fail to show a diurnal decrease.

- They can be a normal/appropriate response to a major stressor.
- High flat slopes might also suggest a challenge that seems insurmountable.

Timed Cortisol Measurements

Specific cortisol elevations throughout a diurnal rhythm may be caused by any number of acute mental, emotional and physical daily stressors, blood sugar dysregulation, exercise or pain. Abnormal results should be correlated with each patient's clinical presentation and specific daily routine.

Morning (7:00 AM – 9:00 AM) cortisol measurement reflects peak ACTH-mediated adrenal gland response.

- Exaggerated levels can be seen with exercise, blood sugar dysregulation, daily stressors, pain, and underlying adrenal hyperplasia or Cushing's syndrome.
- Low levels may reflect an inability to mount a peak response as is seen in adrenal dysfunction and/or down regulation from chronic stressors.

Mid-morning (11:00 AM – 1:00 PM) cortisol levels reflect an adaptive function of the HPA axis to daily routine.

- Elevated levels should be correlated with daily stressors, such as exercise, blood sugar dysregulation, perceived and actual lifestyle stressors and pain.
- Lower levels can reflect HPA axis dysfunction.

Afternoon (3:00 PM – 5:00 PM) cortisol is often reflective of glycemic control due to the post-prandial timing of collection.

Commentary

- Elevated levels can reflect any number of daily stressors as previously outlined.
- Low levels can reflect underlying HPA axis dysfunction.

Evening (10:00 PM – 12:00 AM) cortisol levels are a good indication of baseline HPA axis function since they represent the lowest level during the circadian rhythm.

- Elevated levels may be due to stress, exercise, alcohol, and specific lifestyle stressors.
- Elevated evening salivary cortisol is linked to insomnia
- High evening cortisol levels are also associated with various diseases such as diabetes, cardiovascular disease, hormonally driven cancers, and osteoporosis.

Treatment of elevated cortisol should be directed at the root cause of the stressor. Lifestyle modification with relaxation methods, dietary changes, pain management, and overall HPA axis support with nutrition and/or adaptogens can be helpful. Glandulars may be added if additional support is necessary.

References:

1. Clow A, Thorn L, Evans P. Hucklebridge F. The awakening cortisol response: methodological issues and significance. *Stress.* 2004;7(1):29-37.
2. Stalder T, Kirschbaum C, Kudielka BM, et al. Assessment of the cortisol awakening response: Expert consensus guidelines. *Psychoneuroendocrino.* 2016;63:414-432.
3. Wust S, Wolf J, Hellhammer DH, Federenko I, Schommer N, Kirschbaum C. The cortisol awakening response-normal values and confounds. *Noise health.* 2000;2(7):79.
4. Fries E, Dettenborn L, Kirschbaum C. The cortisol awakening response (CAR): facts and future directions. *IntJPsychophysiol.* 2009;72(1):67-73.
5. Saxbe DE. A field (researcher's) guide to cortisol: tracking HPA axis functioning in everyday life. *Health Psychol Rev.* 2008;2(2):163-190.

CHECKLIST (PRIOR TO SHIPPING)

1. All Tubes

- ❑ **Patient's First and Last Name, Date of Birth, and Collection Start Time and Stop Time** written on all tube labels
- ❑ The specimen **reaches** the FILL LINES in all tubes
  - **3 ml** – White-top tubes
  - **1 ml** – Blue-top tubes
- ❑ All the tubes are **tightly closed**

2. Tubes

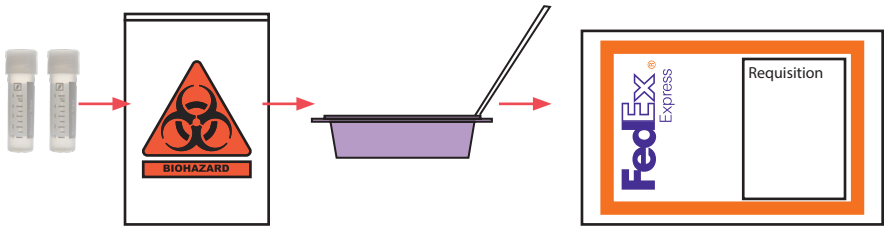
- ❑ All Tubes - frozen

3. Test Requisition Form with Payment

- ❑ Test Requisition Form is complete – **Test is marked, patient's first and last name, date of birth, gender,** and **time collection ended** are recorded
- ❑ **Payment** is included or pay online at **www.gdx.net/prc**

4. Return to the Laboratory

- ❑ Please place samples in biohazard bag, then place biohazard bag in clamshell container. Place container in mailing envelope with requisition. No need to send plastic tray.



SHIP THE SPECIMEN(S) TO THE LAB

Please refer to the shipping instruction insert found in your kit box.

GENOVA DIAGNOSTICS

Call **800.522.4762** or visit our website at **www.gdx.net**

RHYTHM PLUS PROFILE

PATIENT SALIVA COLLECTION INSTRUCTIONS

Rhythm Plus™

The following test(s) can be collected using these instructions:

Rhythm Plus™* **#4102**

Add-on
Cortisol Awakening Response (CAR)* **#4309**

** Not available in New York*

Test may not be processed without this information:

Test Requisition Form

Please Provide:
- ❑ **Patient's First/last Name**
- ❑ **Date of Birth**
- ❑ **Gender**
- ❑ **Date of Collection**

All Tubes

Please Label:
- ❑ **Patient's first/last name**
- ❑ **Patient's date of birth**
- ❑ **Collection date**
- ❑ **Collection start/stop time**

Please read and follow instructions completely to ensure accurate results.

Specimen

Saliva

Additional Materials

- Biohazard bag with absorbent material
- Test Requisition Form
- Collection labels
- Prepaid mailing envelope

Collection Materials for Saliva

2 Blue-top Collection tubes

16 White-top Collection tubes

IMPORTANT PREP PRIOR TO TESTING

IMPORTANT:

- ❑ Your **collection schedule is based on the first day of your menstrual cycle** (the day menstrual blood flow begins). If you are not experiencing periods, you may start on any day. You may find it helpful to **use the enclosed collection calendar** to remind you of collection dates.
- ❑ Consider waking at **6am** on **last day** of collection.
- ❑ If you have difficulty producing enough saliva for the tube, press the tip of your tongue to the roof of your mouth against your teeth. Yawning can also generate saliva.
- ❑ **Transdermal (cream)** and sublingual bio-identical hormones may produce artificially high levels in the saliva that do not correlate with blood levels. This increase from cream hormones may last for weeks to months after discontinuing use. If you are taking these substances – or have taken them within the last 12 months – please consult with your healthcare practitioner before taking this test.
- ❑ The **following drugs, herbs and dietary supplements may influence levels of hormones reported in this test**: ketoconazole, cimetidine (Tagamet), anastrozole (Arimidex), letrozole (Femara), exemestane (Aromasin), Chrysin, Apigenin, Tribulus terrestris, clomiphene, antiepileptics, digoxin, oral steroids (e.g. Prednisone), cortisone cream, and any steroid-based nasal sprays, inhalers, or eye drops. Let your physician know about these and any other medications, herbs, and supplements that you have used in the past 3 months. Do not change use of supplements or medications unless instructed to do so by your healthcare provider.

IMPORTANT:

NIGHT BEFORE COLLECTION:

- ❑ Before you go to sleep on Collection Day, place your collection tube (with a completed label) at your bedside, along with a glass of water and a low level light. Do not turn on a bright light, it will cause your melatonin level to drop.

ONE HOUR BEFORE COLLECTION:

- ❑ One hour prior to collection do not eat, brush or floss your teeth, use mouthwash, chew gum or use any tobacco products. You may drink ONLY water during this time.

For full details refer to: www.gdx.net/tests/prep

Please refer to your requisition for the testing option ordered by your clinician. Pay close attention to the collection times and amount of saliva required. Failure to do so may cause samples to be rejected or alter results.

Adrenocortex Stress Profile **Labels 12-16**

with Cortisol Awakening Response**:** **Labels 12-18**

PLEASE USE THE 28 DAY COLLECTION CALENDAR FOR TUBES 1 -11

COLLECTION

IMPORTANT: To ensure accurate test results you MUST provide the requested information.

1. **Write patient's first and last name, date of birth, gender, and dates of collection** on the Test Requisition Form.

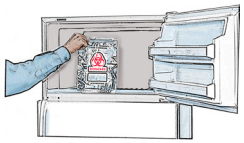
Collecting Your Saliva Samples:

2. **Fill** tube with saliva to designated level, without bubbles or mucus, within 5 minutes. **Replace** the cap tightly to avoid leakage.

3. Please **write** the **patient's first and last name, date of birth, and the start and stop collection times on the label. Attach** the label to the collection tube.

NAME: _____ 1
D.O.B.:___/___/____ DATE: _____
START TIME: _____
STOP TIME: _____

4. **Freeze** tube immediately. Samples must be frozen a minimum of 2 hours prior to shipping. Keep samples frozen until ready to ship.



5. **Repeat** these steps for each sample according to the Specimen Collection Chart.

SPECIMEN COLLECTION CHART

SPECIMEN INTERVALS	ASP	with CAR
WAKING Collect immediately upon waking		1 ml
30 MINUTES Collect 30 minutes from end of waking collection		1 ml
Collect Between **7:00AM – 9:00AM**	3 ml	3 ml
Collect Between **11:00AM – 1:00PM**	3 ml	3 ml
Collect Between **3:00PM – 5:00PM**	3 ml	3 ml
Collect Between **10:00PM – 12:00AM**	3 ml	3 ml
Collect between **2:30AM - 3:30AM**	3 ml	3 ml

Rhythm Plus™

28 Day Collection Calendar

Your collection schedule is based on the first day of your menstrual cycle (day menstrual blood flow begins). Use this calendar to log the following dates to assist your healthcare provider with interpretation of your results: Enter onset date of **PREVIOUS** menstrual period_____; enter onset date of **CURRENT** menstrual period & all collection dates in the chart below; and onset date of **NEXT** menstrual period _____ (onset date of menstrual period following the completion of this saliva test).

**Note: this calendar is meant to serve as a guide to aid in your collection. See full kit instructions for details on acceptable specimen collection.*

Insert the days of week starting with the first day in which your menstrual cycle began. ie. **Tues, Weds, Thurs,** etc.

DAY 1	**DAY 2**	**DAY 3**	**DAY 4**	**DAY 5**	**DAY 6**	**DAY 7**
Do not collect First day of your menstrual cycle		**1st Collection** 7-9 AM Label #1		**2nd Collection** 7-9 AM Label #2		
DATE:	DATE:	DATE:	DATE:	DATE:	DATE:	DATE:
DAY 8	**DAY 9**	**DAY 10**	**DAY 11**	**DAY 12**	**DAY 13**	**DAY 14**
3rd Collection 7-9 AM Label #3			**4th Collection** 7-9 AM Label #4	**5th Collection** 7-9 AM Label #5		**6th Collection** 7-9 AM Label #6
DATE:	DATE:	DATE:	DATE:	DATE:	DATE:	DATE:
DAY 15	**DAY 16**	**DAY 17**	**DAY 18**	**DAY 19**	**DAY 20**	**DAY 21**
	7th Collection 7-9 AM Label #7		**8th Collection** 7-9 AM Label #8		**9th Collection** 7-9 AM Label #9	
DATE:	DATE:	DATE:	DATE:	DATE:	DATE:	DATE:
DAY 22	**DAY 23**	**DAY 24**	**DAY 25**	**DAY 26**	**DAY 27**	**DAY 28**
	10th Collection 7-9 AM Label #10					**11th Collection** 7-9 AM Label #11
DATE:	DATE:	DATE:	DATE:	DATE:	DATE:	DATE:

USE THE COLLECTION SCHEDULE WITHIN THE SPECIMEN COLLECTION INSTRUCTIONS FOR DAY 29