

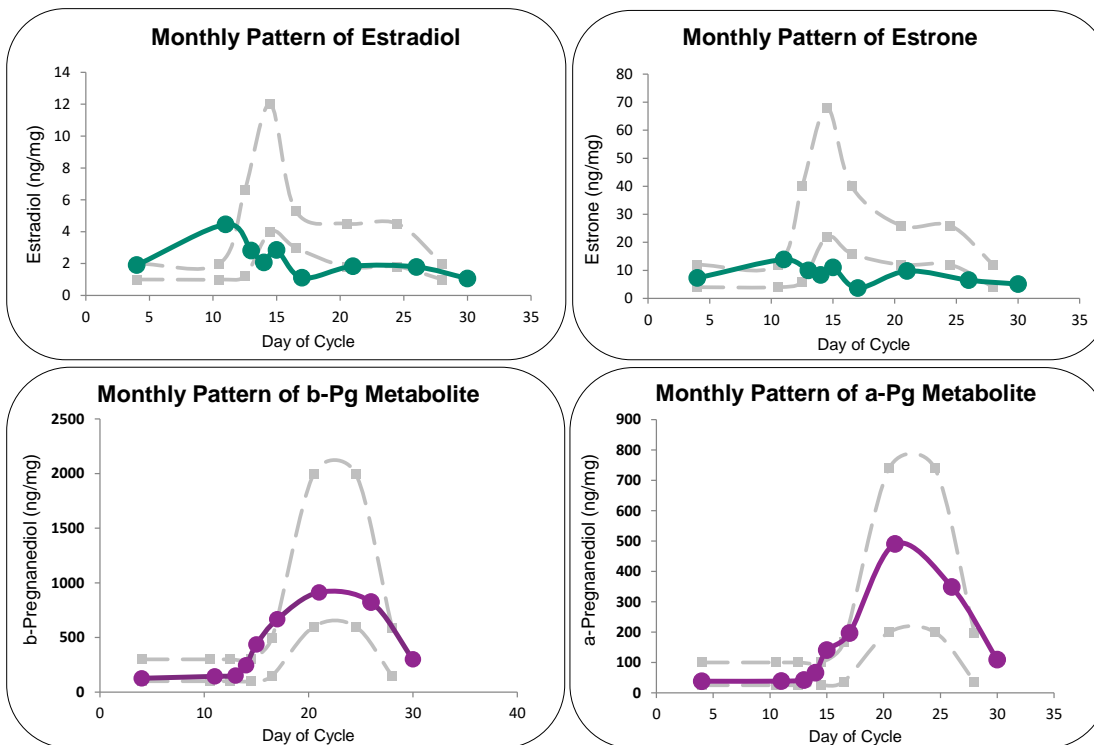
# Cycle Mapping Results



Name: Sample Report  
 Provider: Regenerus Laboratories Ltd  
 Accession #

D.O.B.  
 Collection Dates  
 12/10/2021-1/1/2022

Estrogen (E) patterns can be seen below in green. Progesterone (Pg) patterns can be seen below in purple. Normal ranges are within the gray dashed lines. See page 2 for more information.



All values given in ng/mg creatinine

Measurement	1	2	3	4	5	6	7	8	9
Day(s) of Cycle	4	11	13	14	15	17	21	26	30

The days listed above were used for measurements. Two samples are used and listed for long cycles or patients without a normal cycle.

Estradiol (E2)	1.91	4.46	2.81	2.07	2.85	1.11	1.83	1.79	1.06
Estrone (E1)	7.3	13.9	10.0	8.4	11.1	3.7	9.7	6.5	5.1
a-Pregnanediol	38	38	42	66	141	197	490	349	109
b-Pregnanediol	127	144	153	245	437	667	913	822	301
b-Pregnanediol/E2 Ratio	67	32	54	118	153	603	498	461	283
Creatinine		0.42	1.22	1.15	0.59	1.40	0.83	1.28	0.64

Sample (#7) with the highest b-Pg value (913) is used for E and Pg metabolites for DUTCH Complete or Plus if ordered.

Normal Ranges	Follicular	Ovulatory	Luteal	Postmenopausal
Estradiol	1-2ng/mg	4-12ng/mg	1.8-4.5ng/mg	0.2-0.7ng/mg
Estrone	4-12ng/mg	22-68ng/mg	12-26ng/mg	1.0-7.0ng/mg
a-Pregnanediol	25-100ng/mg	25-100ng/mg	200-740ng/mg	15-50ng/mg
b-Pregnanediol	100-300ng/mg	100-300ng/mg	600-2000ng/mg	60-200ng/mg

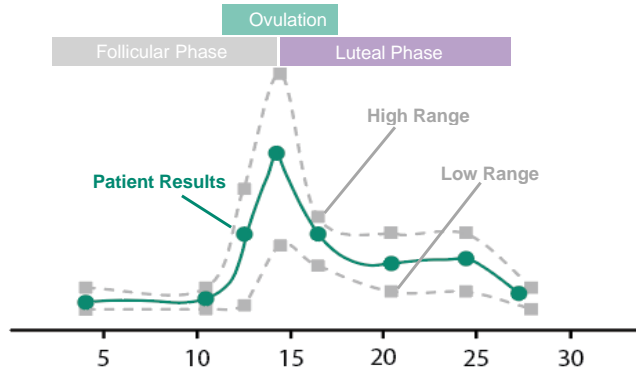
b-Pregnanediol/E2 ratio is typically 50-300 in the follicular phase, <100 during ovulation, and 100-500 in the luteal phase. Creatinine normal range, 0.2-2.0 mg/mL. Values outside this range may be less certain due to under or overhydration.

# Cycle Mapping Guide



Thank you for testing with us! If this is your first report, you are encouraged to watch our educational videos on how to read the report at [www.dutchtest.com](http://www.dutchtest.com) in the [video library](#). The comments below include general information that we hope you will find useful in your understanding of the patient's results. These results and comments are not intended to diagnose any specific conditions.

You'll find four stacked graphs with the reference ranges and the patient's results mapped out. The top graphs represent estrogen (E) production, and the bottom graphs represent progesterone (Pg) production. The horizontal axis shows the cycle days (0-30+) and the vertical axis shows hormone concentration or hormone metabolites being measured. Healthy cycles typically range from 21-35 days. The patient likely submitted many samples over one cycle, and we have selected the 9 most relevant measurements. Some measurements from longer cycles are from two-day averages to ensure transitory E and Pg peaks are not missed.

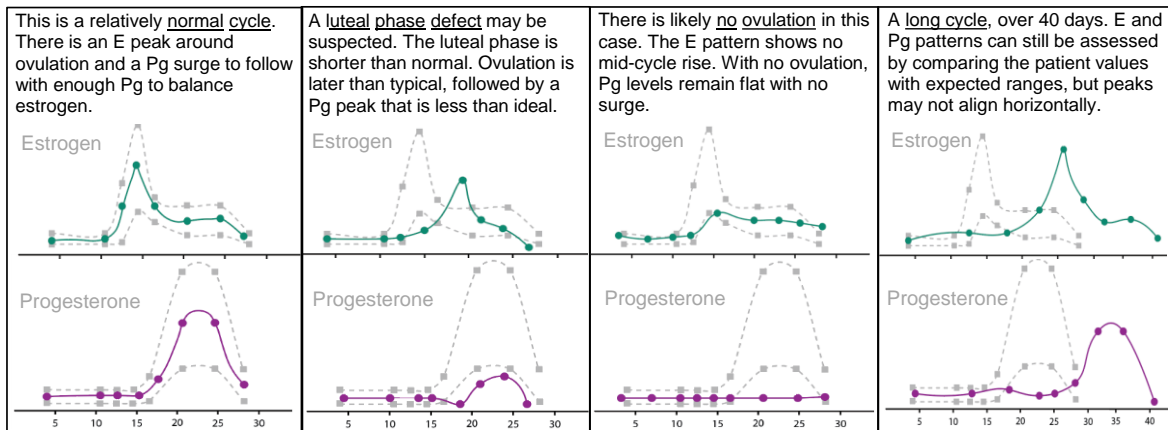


For most cycles <34 days, measurements are made from single days, selected to best represent overall patterns of ovulatory & luteal peaks. The day 4 sample set is usually collected at the end of the cycle, four days after menses (used for DUTCH Complete/Plus), but is plotted at the beginning of the cycle as above. If a DUTCH Complete or Plus was ordered, data for the E and Pg metabolite values are taken from the day on the Cycle Mapping associated with the progesterone (b-pregnanediol) peak in the luteal phase.

The first part of the cycle (days 1-14) is the "follicular phase," ovulation typically occurs mid-cycle, and the "luteal phase" refers to the 2<sup>nd</sup> half of the cycle (days 14 until menses). These phases may shift in patients with atypical cycle lengths. Levels may still be considered normal in short or long cycles even if the timing of the E or Pg peaks are at different times.

In the top graphs, we follow both primary estrogens, estrone (E1) and estradiol (E2). In a typical cycle, estrogen rises in the follicular phase, which stimulates the luteinizing hormone (LH) surge from the brain about 24-36 hours before ovulation, which leads to the production of Pg the second half of the cycle (measured by its primary pregnanediol metabolites). Pg rises only after ovulation has occurred, reaching its peak 5-7 days later, then begins to decrease before the onset of menses. When Pg does not rise it indicates that the patient is likely not ovulating. A weak rise in Pg can also indicate either no ovulation or a weak corpus luteum (luteal phase defect), which is associated with poor egg maturation, difficulty maintaining a secretory endometrium and infertility. Ranges for Pg are similar for a postmenopausal woman or a cycling woman who is in the follicular phase. In the table near the bottom of page 1 below the graphs, the patient's results are displayed in a table. This includes creatinine, which is used to correct for hydration. If creatinine is very low or very high, hormone measurements from that day may be less reliable.

Below are four different cycle patterns that may help with interpretation ([video tutorial here](#))



**Age:** 45  
**Sex:** Female

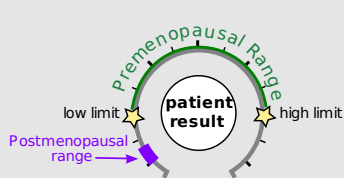
**Last Menstrual Period:**

**Ordering Provider:**  
Regenerus Laboratories Ltd

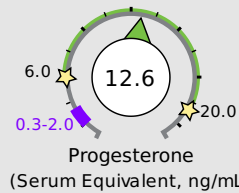
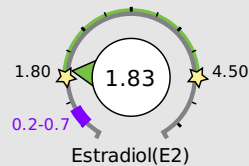
**Collection Times:**  
2022-01-01 07:20AM  
2022-01-01 09:20AM  
2022-01-01 05:20PM  
2022-01-01 10:20PM

## Hormone Testing Summary

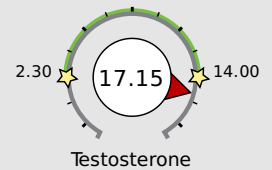
### Key (how to read the results):



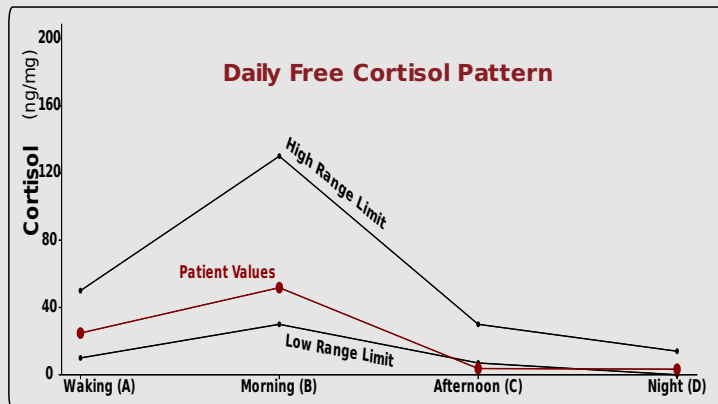
### Sex Hormones See Pages 2 and 3 for a thorough breakdown of sex hormone metabolites



Progesterone Serum Equivalent is a calculated value based on urine pregnanediol.



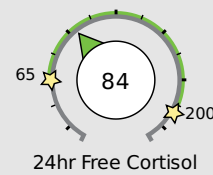
### Adrenal Hormones See pages 4 and 5 for a more complete breakdown of adrenal hormones



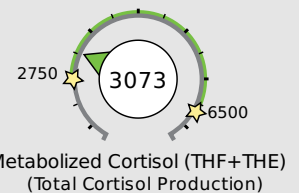
Free cortisol best reflects tissue levels. Metabolized cortisol best reflects total cortisol production.

### Total DHEA Production

Age	Range
20-39	1300-3000
40-60	750-2000
>60	500-1200



cortisol  
metabolism



The following videos (which can also be found on the website under the listed names along with others) may aid your understanding:

[DUTCH Complete Overview](#) [Estrogen Tutorial](#) [Female Androgen Tutorial](#) [Cortisol Tutorial](#)

**PLEASE BE SURE TO READ BELOW FOR ANY SPECIFIC LAB COMMENTS. More detailed comments can be found on page 7.**

- The patient mentioned taking oral or sublingual DHEA and DHEA metabolite results are elevated. If the patient did not skip the dose the day of the test (as instructed), results may be artificially elevated from first-pass metabolites. Please see requisition or discuss with patient to confirm.



Accession #



**Sex Hormones and Metabolites**

**DOB:**

**Age:** 45

**Sex:** Female

**Last Menstrual Period:**

**Ordering Provider:**

Regenerus Laboratories Ltd

**Collection Times:**

2022-01-01 07:20AM

2022-01-01 09:20AM

2022-01-01 05:20PM

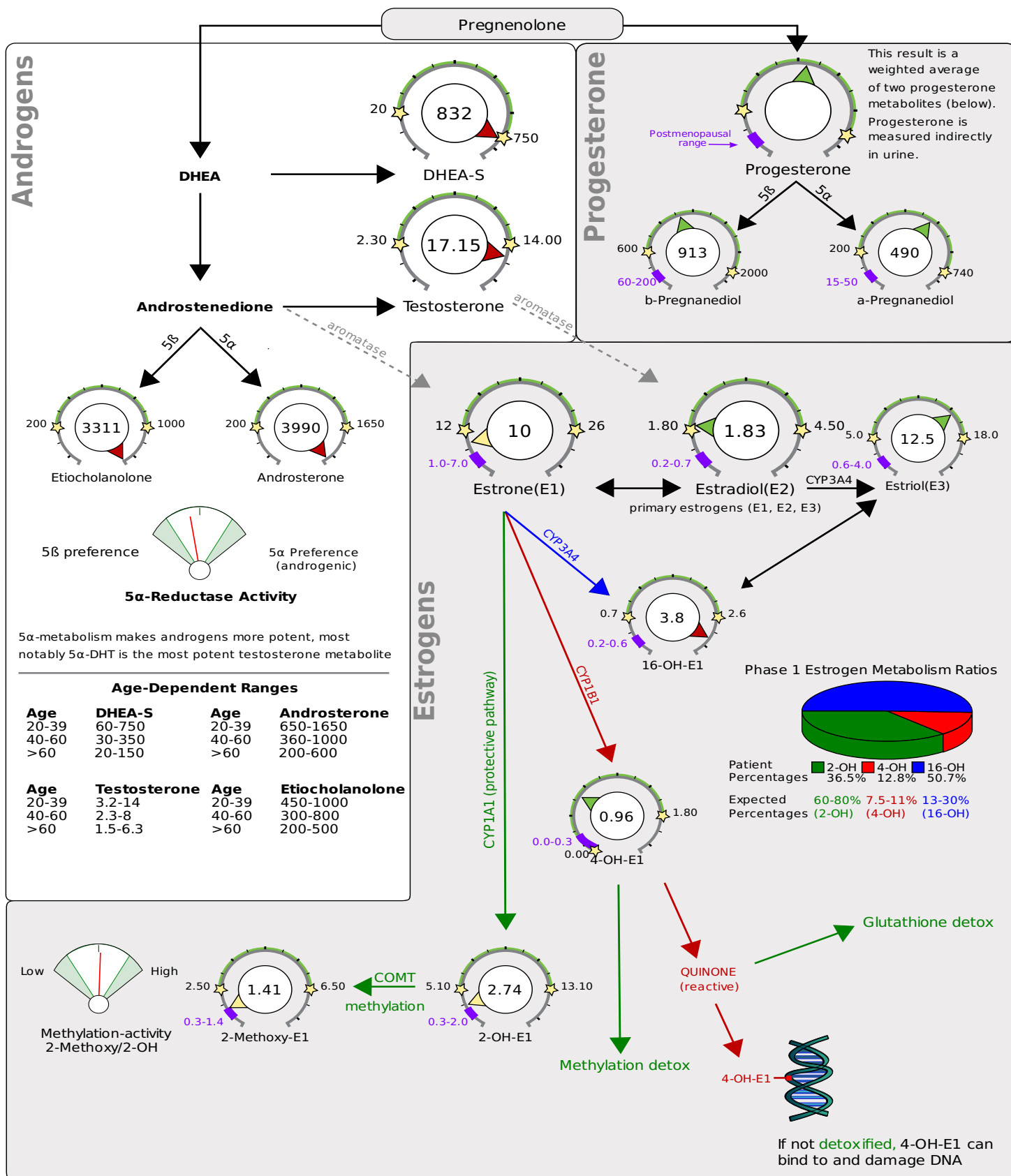
2022-01-01 10:20PM

Test		Result	Units	Luteal*	Postmenopausal
Progesterone Metabolites (Urine)				Range	Range
b-Pregnanediol	Within luteal range	912.9	ng/mg	600 - 2000	60-200
a-Pregnanediol	Within luteal range	490.1	ng/mg	200 - 740	15-50
Estrogens and Metabolites (Urine)					
Estrone(E1)	Below luteal range	9.75	ng/mg	12 - 26	1.0-7.0
Estradiol(E2)	Low end of luteal range	1.83	ng/mg	1.8 - 4.5	0.2-0.7
Estriol(E3)	Within luteal range	12.5	ng/mg	5 - 18	0.6-4.0
2-OH-E1	Below luteal range	2.74	ng/mg	5.1 - 13.1	0.3-2.0
4-OH-E1	Within luteal range	0.96	ng/mg	0 - 1.8	0-0.3
16-OH-E1	Above luteal range	3.8	ng/mg	0.7 - 2.6	0.2-0.6
2-Methoxy-E1	Below luteal range	1.41	ng/mg	2.5 - 6.5	0.3-1.4
2-OH-E2	Low end of luteal range	0.19	ng/mg	0 - 1.2	0-0.3
4-OH-E2	Within luteal range	0.16	ng/mg	0 - 0.5	0-0.1
Total Estrogen	Below range	33.4	ng/mg	35 - 70	4.0-15
Androgens and Metabolites (Urine)					
DHEA-S	Above range	831.7	ng/mg	20 - 750	
Androsterone	Above range	3989.8	ng/mg	200 - 1650	
Etiocholanolone	Above range	3310.9	ng/mg	200 - 1000	
Testosterone	Above range	17.15	ng/mg	2.3 - 14	
5a-DHT	Above range	11.9	ng/mg	0 - 6.6	
5a-Androstanediol	Above range	144.1	ng/mg	6 - 30	
5b-Androstanediol	Above range	185.8	ng/mg	20 - 75	
Epi-Testosterone	Low end of range	2.5	ng/mg	2.3 - 14	

\*the Luteal Range is the premenopausal range. When patients are taking oral progesterone this range for progesterone metabolites is not luteal and reflects the higher levels expected when patients take oral progesterone. This test is intended to be taken in the luteal phase of the menstrual cycle (days 19-22 of a 28 day cycle) for premenopausal women. The ranges in the table below may be used when samples are taken during the first few days (follicular) of the cycle, during ovulation (days 11-14) or when patients are on oral progesterone. See the following pages for age-dependent ranges for androgen metabolites.

Additional Normal Ranges	Follicular	Ovulatory	Oral Pg (100mg)
b-Pregnanediol	100-300	100-300	2000-9000
a-Pregnanediol	25-100	25-100	580-3000
Estrone (E1)	4.0-12.0	22-68	N/A
Estradiol (E2)	1.0-2.0	4.0-12.0	N/A

Hormone metabolite results from the previous page are presented here as they are found in the steroid cascade. See the Provider Comments for more information on how to read the results.





## Accession



### Adrenal

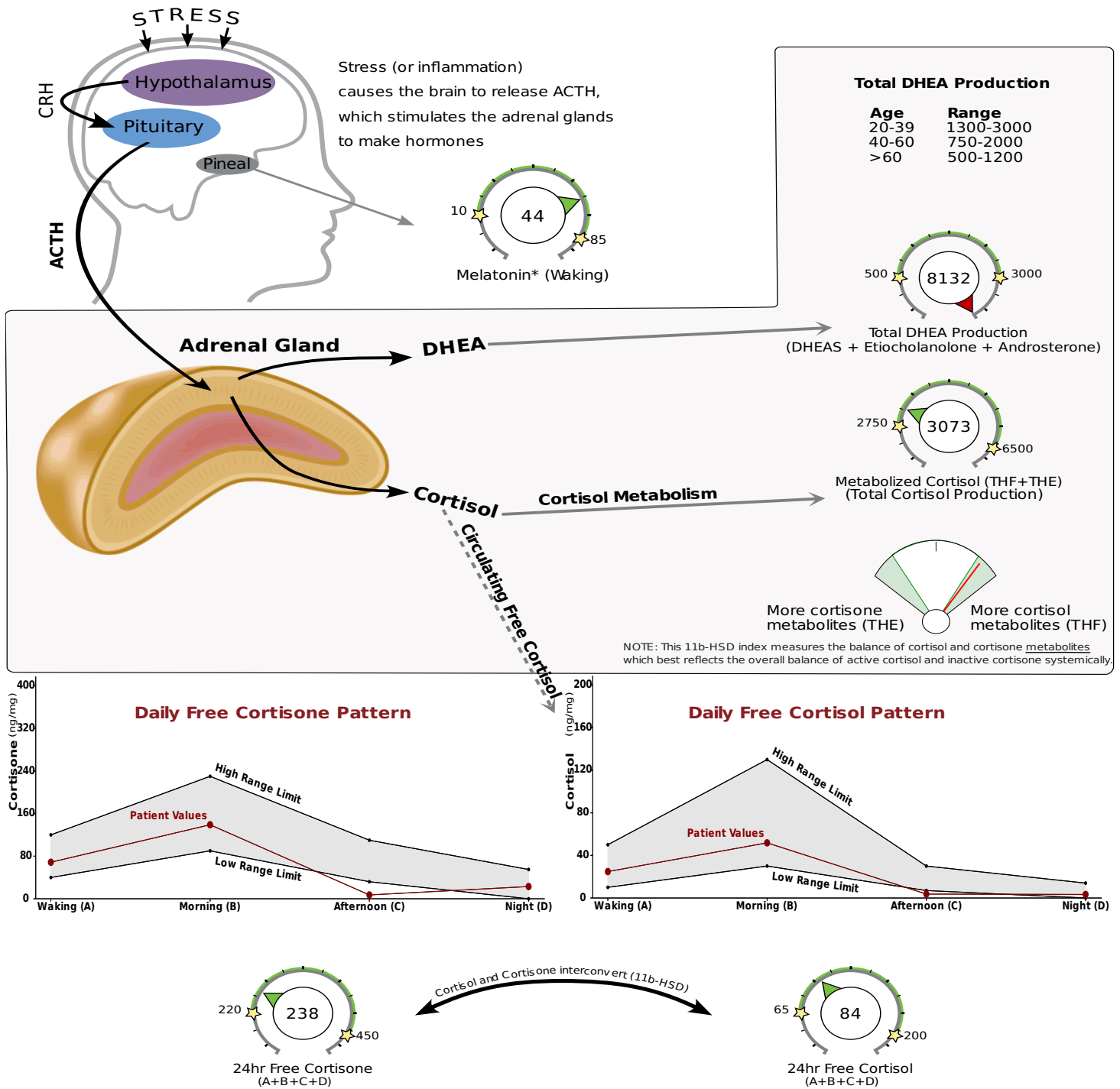
DOB:  
Age: 45  
Sex: Female

### Last Menstrual Period:

Ordering Provider:  
Regenerus Laboratories Ltd

Collection Times:  
2022-01-01 07:20AM  
2022-01-01 09:20AM  
2022-01-01 05:20PM  
2022-01-01 10:20PM

Category	Test		Result	Units	Normal Range
<b>Creatinine (Urine)</b>					
	Creatinine A (Waking)	Within range	0.51	mg/ml	0.2 - 2
	Creatinine B (Morning)	Within range	0.38	mg/ml	0.2 - 2
	Creatinine C (Afternoon)	Within range	0.46	mg/ml	0.2 - 2
	Creatinine D (Night)	Within range	0.63	mg/ml	0.2 - 2
<b>Daily Free Cortisol and Cortisone (Urine)</b>					
	Cortisol A (Waking)	Within range	24.8	ng/mg	10 - 50
	Cortisol B (Morning)	Within range	51.8	ng/mg	30 - 130
	Cortisol C (Afternoon)	Below range	3.7	ng/mg	7 - 30
	Cortisol D (Night)	Within range	3.3	ng/mg	0 - 14
	Cortisone A (Waking)	Within range	68.6	ng/mg	40 - 120
	Cortisone B (Morning)	Within range	139.0	ng/mg	90 - 230
	Cortisone C (Afternoon)	Below range	7.2	ng/mg	32 - 110
	Cortisone D (Night)	Within range	23.0	ng/mg	0 - 55
	24hr Free Cortisol	Low end of range	83.7	ng/mg	65 - 200
	24hr Free Cortisone	Low end of range	237.8	ng/mg	220 - 450
<b>Cortisol Metabolites and DHEA-S (Urine)</b>					
	a-Tetrahydrocortisol (a-THF)	Low end of range	107.9	ng/mg	75 - 370
	b-Tetrahydrocortisol (b-THF)	Within range	1459.7	ng/mg	1050 - 2500
	b-Tetrahydrocortisone (b-THE)	Below range	1505.5	ng/mg	1550 - 3800
	Metabolized Cortisol (THF+THE)	Low end of range	3073.1	ng/mg	2750 - 6500
	DHEA-S	Above range	831.7	ng/mg	20 - 750



The first value reported (Waking "A") for cortisol is intended to represent the "overnight" period. When patients sleep through the night, they collect just one sample. In this case, the patient did not report waking up during the night to collect a sample, so the "Waking (A)" cortisol and cortisone values should accurately represent the entirety of the overnight period.





## Accession



### Organic Acid Tests (OATs)

**Ordering Provider:**  
Regenerus Laboratories Ltd

**DOB:**  
**Age:** 45  
**Sex:** Female

### Last Menstrual Period:

**Collection Times:**  
2022-01-01 07:20AM  
2022-01-01 09:20AM  
2022-01-01 05:20PM  
2022-01-01 10:20PM

Category	Test	Result	Units	Normal Range
<b>Nutritional Organic Acids</b>				
Vitamin B12 Marker (may be deficient if high) - (Urine)				
	Methylmalonate (MMA)	Within range	1.4	ug/mg 0 - 2.5
Vitamin B6 Markers (may be deficient if high) - (Urine)				
	Xanthurenate	Within range	0.93	ug/mg 0.12 - 1.2
	Kynurenate	Within range	2.6	ug/mg 0.8 - 4.5
Glutathione Marker (may be deficient if low or high) - (Urine)				
	Pyroglutamate	Within range	36.6	ug/mg 28 - 58
<b>Neurotransmitter Metabolites</b>				
Dopamine Metabolite - (Urine)				
	Homovanillate (HVA)	Within range	6.0	ug/mg 3 - 11
Norepinephrine/Epinephrine Metabolite - (Urine)				
	Vanilmandelate (VMA)	Within range	2.9	ug/mg 2.2 - 5.5
Melatonin (*measured as 6-OH-Melatonin-Sulfate) - (Urine)				
	Melatonin* (Waking)	Within range	44.4	ng/mg 10 - 85
Oxidative Stress / DNA Damage, measured as 8-Hydroxy-2-deoxyguanosine (8-OHdG) - (Urine)				
	8-OHdG (Waking)	Within range	3.7	ng/mg 0 - 5.2



# Clinical Support Overview

Thank you for choosing DUTCH for your functional endocrinology testing needs! We know you have many options to choose from when it comes to functional endocrinology evaluation, and we strive to offer the best value, the most up-to-date testing parameters and reference ranges, and the greatest clinical support to ensure the most accurate results.

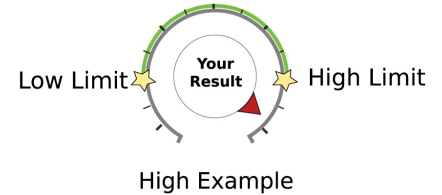
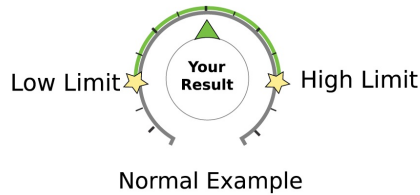
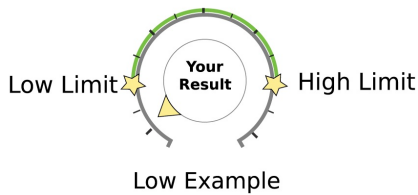
Please take a moment to read through the Clinical Support Overview below. These comments are specific to the patient's lab results. They detail the most recent research pertaining to the hormone metabolites, treatment considerations, and follow-up recommendations. These comments are intended for educational purposes only. Specific treatment should be managed by a healthcare provider. To view the steroid pathway chart, click here [Steroid Pathway Chart](#)

## Alert comments:

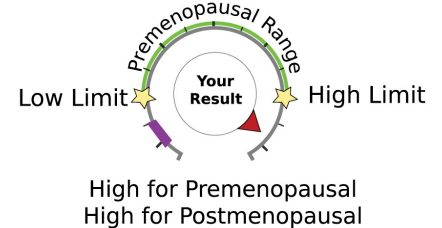
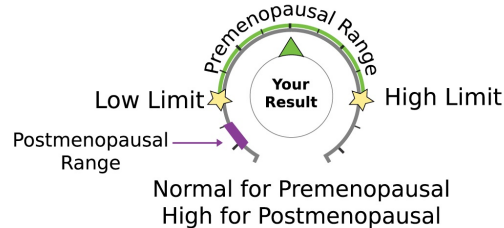
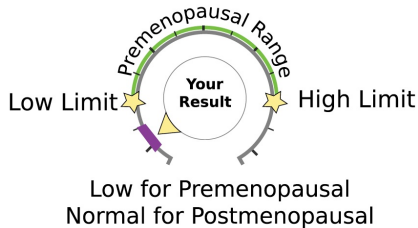
**- This DUTCH Test is associated with a Cycle Mapping report as well. The progesterone and estrogen metabolites for this report are based on the Cycle Mapping sample with the highest b-pregnenediol (primary progesterone metabolite) value.**

## How to read the DUTCH report

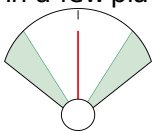
This report is not intended to treat, cure or diagnose any specific diseases. The graphic dutch dials in this report are intended for quick and easy evaluation of which hormones are out of range. Results below the left star are shaded yellow and are below range (left). Results between the stars and shaded green are within the reference range (middle). Results beyond the second star and shaded red are above the reference range (right). Some of these hormones also change with age, and the age-dependent ranges provided should also be considered.



For female reproductive hormones, a purple band is present on the dutch dials. This band represents the expected levels (reference range) for postmenopausal (or non-cycling) women.



In a few places on the graphical pages, you will see fan-style gauges. For sex hormones, you will see one for the balance between 5a/5b metabolism as well as methylation. For adrenal hormones, you will see one to represent the balance between cortisol and cortisone metabolites. These indexes simply look at the ratio of hormones for a preference. An average or "normal" ratio between the two metabolites (or groups of metabolites) will give a result in the middle (as shown here). If the ratio between the metabolites measured is "low" the gauge will lean to the left and similarly to the right if the ratio is higher than normal.



## Patient or Sample Comments

Throughout the provider comments you may find some comments specific to your situation or results. These comments will be found in this section or within another section as appropriate. Comments in other sections that are specific to your case will be in **bold**.

**The patient reports regular menstrual cycles.**

**The patient reported significant fatigue in the afternoon/evening, but not in the morning.**

## Progesterone Metabolism

Progesterone is made predominately in the ovaries by the corpus luteum following the release of an egg. Progesterone metabolite levels will increase to the premenopausal luteal range (the range established as the green band between the two gold stars) only after the release of an egg. The level of progesterone metabolites seen on the DUTCH test can help determine if ovulation occurred 5-7 days prior to test collection.

The primary role of progesterone is to prepare the endometrium of the uterus for implantation. In addition, it may balance the effects of estrogen, it is a neurosteroid, it acts as a diuretic and raises basal body temperature.

We are measuring metabolites of progesterone 5b-pregnanediol and 5a-pregnanediol. 5b-pregnanediol has less activity in the body but does represent a larger percent of total progesterone metabolism overall. 5a-pregnanediol is often a metabolite of more interest, as it can cross the blood brain barrier and up-regulate GABA activity and is considered neuroprotective to the brain. In some women the 5a-pregnanediol is also the cause of PMDD and irritability due to issues with the GABA receptor's inability to adjust for sensitivity to fluctuating neurosteroids (Dr Briden).

If progesterone levels are in the low or lower end of the luteal reference range compared to estrogen levels, women may experience symptoms such as PMS, menorrhagia, mastalgia, moodiness, anxiety, and/or insomnia.

The metabolites of progesterone are excreted in urine (not the progesterone itself). When ordering the DUTCH Complete and DUTCH Plus reports, you will see a Progesterone Serum Equivalent on the summary page 1. The urine metabolites of progesterone have been proven to correlate strongly to serum progesterone. The Progesterone Serum Equivalent is most accurate with values in the luteal range and becomes more approximate at very low numbers in the postmenopausal range. Cycling women with very high progesterone metabolites may also decrease the accuracy of the serum equivalent calculation.

NOTE: If progesterone is taken orally (also with sublingual), these metabolites are elevated from gut metabolism and results do NOT accurately reflect serum levels.

**Progesterone metabolites are in range for a cycling woman in the luteal phase of the menstrual cycle, indicating ovulation likely occurred 5-7 days before the sample collection. Evaluate if healthy progesterone levels are in balance with estrogen, especially if cyclical symptoms are a part of the picture.**

**Of the two progesterone metabolites, the patient favors the a-Pregnanediol metabolite. This metabolite can cross the blood-brain-barrier and works on GABA receptors, giving a calming effect. Women who metabolize their progesterone through this pathway tend to have more significant benefit from oral progesterone for symptoms of anxiety and difficulty sleeping.**

### Estrogen Metabolism

As of 12.3.2021 we have discontinued 2-methoxy-E2. After careful consideration of measurements of both 2-methoxy-E2 as well as 4-methoxy-E1, we have determined that the relationship between 2-OH-E1 and 2-methoxy-E1 is the best way to determine methylation of catechol estrogen metabolites. Our decision reflects our commitment to focusing on the most reliable laboratory measurements when making clinical decisions.

When evaluating estrogen levels, it is important to assess the following:

- **The status (low, normal or high?) of estrogen production:**

Levels of the primary ovarian product, estradiol (the strongest estrogen), as well as "total estrogens" may be considered. For women not on HRT, consider the appropriate range (premenopausal or postmenopausal).

- **Phase I Metabolism:**

Estrogen is metabolized (primarily by the liver) down three phase I pathways. The 2-OH pathway is considered the safest because of the anti-cancer properties of 2-OH metabolites. Conversely, the 4-OH pathway is considered the most genotoxic as its metabolites can create reactive products that damage DNA. The third pathway, 16-OH creates the most estrogenic of the metabolites (although still considerably less estrogenic than estradiol) - 16-OH-E1. If overall estrogen levels are high, production of 16-OH-E1 may exacerbate high estrogen symptoms. Similarly, a woman with very low levels of estrogens, may have less low estrogen symptoms if 16-OH metabolism is preferred. For example Armamento-Villareal showed that a higher 2-OH-E1/16-OH-E1 ratio correlated to bone loss (a low estrogen symptom). Estriol is thought of as a safer (weaker) estrogen metabolite, but it is important to remember that estriol is actually 16-OH-E2, so generally patients that make a lot of the potentially protective/weak estriol may also make a lot of the estrogenic 16-OH-E1.

When evaluating phase I metabolism, it may be important to look at the ratios of the three metabolites to see which pathways are preferred relative to one another. It may also be important to compare these metabolites to the levels of the parent hormones (E1, E2). If the ratios of the three metabolites are favorable but overall levels of metabolites are much lower than E1 and E2, this may imply sluggish phase I clearance of estrogens, which can contribute to high levels of E1 and E2. Similarly, patients with excessive phase I metabolism may have low E1 and E2 levels because of high rates of clearance (as opposed to simply not making a lot of estrogen). The pie chart will assist you in comparing the three pathway options of phase I metabolism compared to what is "normal." 2-OH metabolism can be increased by using products containing D.I.M. or I-3-C. These compounds are found (or created from) in cruciferous vegetables and are known for promoting this pathway.

**Patients typically metabolize a much higher percentage of their estrogens down the more protective 2-OH pathway in phase 1 detoxification. Diindolylmethane (DIM) or Indole-3-Carbinol containing products can help move estrogens more efficiently down this pathway. Be aware that this typically lowers most of the other estrogens, including E1 and E2 as well. If the patients are taking or considering hormone replacement therapy, these products may be considered but a higher dose of estrogen may be needed for the same clinical effect if taken at the same time.**

- **Methylation (part of phase II metabolism) of estrogens:**

After phase I metabolism, both 4-OH and 2-OH (not 16-OH) estrogens can be deactivated and eliminated by

methylation. The methylation-activity index shows the patient's ratio of 2-Methoxy-E1 / 2-OH-E1 compared to what is expected. Low methylation can be caused by low levels of nutrients needed for methylation and/or genetic abnormalities (COMT, MTHFR). The COMT enzyme responsible for methylation requires magnesium and methyl donors. Deficiencies in folate or vitamin B6 or B12 can cause low levels of methyl donors. MTHFR genetic defects can make it more difficult for patients to make sufficient methyl donors. Genetic defects in COMT can make methylation poor even in the presence of adequate methyl donors.

### **Androgen Metabolism**

As of 12.3.2021 the reference range has been updated for 5a-androstanediol for female patients. This change represents improved analytical methods in migrating urine androgens from GC-MS/MS to LC-MS/MS. This improved method has necessitated a change to expected values for this one metabolite.

#### **Androgen Metabolites: DHEA**

DHEA and androstenedione are made almost exclusively by the adrenal gland (although a smaller amount is made in the ovaries for). These hormones appear in urine as DHEA-S (DHEA-Sulfate), androsterone and etiocholanolone.

DHEA peaks for men and women in their 20's and 30's, with a slow decline expected with age. DHEA mainly circulates throughout the body as DHEA-s, with interconversion to active DHEA as it reaches various tissues. DHEA is a weak androgen and will predominately convert to androstenedione, which will then convert to testosterone or estrogen. DHEA-s is made by sulfation, has a much longer half-life than DHEA and largely lacks a diurnal rhythm, which is why it is considered the best way to assess DHEA levels in the body. DHEA-s levels can be affected both by the total production as well as by the body's ability to sulfate DHEA.

The best way to assess the total production of DHEA is to add up these three metabolites. As DHEA production decreases quite significantly with age, we provide the age-dependent ranges. Adrenal DHEA serves as the main source of estrogen, progesterone and testosterone for post-menopausal women.

**The Total DHEA Production (page 1) was 8,132ng/mg which is elevated. High DHEA can cause symptoms of androgen excess including oily skin, acne, sleep problems, headaches and mood disturbances. High levels may be due to supplementation, insulin, stress, elevated prolactin, alcohol and certain medications like ADD meds, Xanax and Wellbutrin. High DHEA can be treated with blood sugar balancing lifestyle, stress reduction and in appropriate cases ashwagandha. In some cases, highly androgenic people may show high levels of both DHEA or testosterone without negative clinical consequence.**

**The patient reports use of oral DHEA. For women, DHEA will often increase both testosterone and estrogen levels. In men the metabolism is sometimes less favorable shifting more towards estrogens than is ideal. You will want to carefully examine androgens and estrogens with oral DHEA therapy. The DHEA measured in urine is primarily DHEA-S. DHEA can increase downstream metabolites significantly without necessarily increasing DHEA-S by the same degree. Free DHEA is not reported but the androstenedione metabolites (androsterone, etiocholanolone) may parallel DHEA levels.**

**Please note that if the patient does not skip the dose the day of and the day before the test, urine results may be elevated by first-pass metabolites. This would overestimate systemic levels. This is why directions ask the patient to skip the dose for 48 hours prior to testing. If results are elevated, be sure the patient followed the directions.**

#### **• Androgen Metabolites: Testosterone**

The DUTCH test measures the total of testosterone glucuronide and testosterone sulfate. These conjugates of testosterone are formed mostly from bioavailable testosterone that undergoes phase 2 metabolism to make it ready for urine excretion. Females make most of their DHEA in the adrenal gland and a fraction of that DHEA trickles down metabolically to testosterone. Testosterone is also made by the ovaries.

Testosterone glucuronide is mostly made by the UGT2B17 enzyme, which also makes the glucuronide forms of 5a-DHT and 5b-androstanediol. Genetic variants of this enzyme reduce the urinary levels of these hormones without affecting serum levels. The genetic variants of UGT2B17 vary in the population from 7-80% (variation dependent on genetic ancestry, with the highest rates in those of Asian descent). Heterozygous individuals show milder reductions in urinary testosterone than homozygous. For this reason, low and very low levels of urinary testosterone should be confirmed with serum testing before treatment is applied. Serum testing can include free and total testosterone and SHBG.

Testosterone levels may be better understood by also considering its downstream metabolites (5 $\alpha$ -androstenediol, 5 $\beta$ -androstenediol). Technically, these metabolites can also be formed from DHEA metabolites without going through the testosterone pathway, but they generally tend to correlate with testosterone production.

Testosterone levels normally decline with age. Age dependent ranges are provided. Perimenopausal testosterone levels can transiently increase before declining again.

Epi-testosterone (epi-T) is made at about the same rate as testosterone but is not androgenic. In cases where testosterone in urine is low, such as with the UGT2B17 deletion discussed above, epi-T may be used as a proxy for testosterone production, meaning that higher epi-T levels may indicate that a low testosterone result is falsely low. After menopause, epi-T production is less reliable as a marker of testosterone production as epi-T levels drop more sharply than does testosterone during the menopause transition. While epi-T may have limited utility in some cases, it does enhance the picture when taking androgen metabolites together as a whole. Androgens, specifically DHT and testosterone, help to support skin, connective tissue, bone and muscle integrity and promote dopamine conversion in the brain, which can help with mood and libido.

**The testosterone level for this patient is high at 17.1ng/mg. In women of any age, high testosterone may be due to emotional or physical stress, as the adrenals are a major source of androgens for women. However, insulin issues, obesity or PCOS are common causes as well. Consider testing and addressing any blood sugar imbalance, source of inflammation and lowering stress depending on the patient's history. Supplements to consider, depending on the root cause, are licorice root, white peony, chaste tree berry, myo-inositol and/or ground flax seed. Medications might include metformin or spironolactone if appropriate.**

**If the patient is post-menopausal or does not have PCOS or metabolic issues, consider exogenous exposure such as from someone in the household using testosterone creams or gels and either touching the sample paper or exposing the patient through skin contact. If downstream metabolites of testosterone (5 $\alpha$ -DHT, 5 $\alpha$ - and 5 $\beta$ - androstenediol) are also elevated, then the high androgens are systemic. If only the testosterone is high and downstream metabolites are normal or low, it may be contamination of the sample paper with a gel or cream rather than systemic. Finally, in some women the epi-testosterone is low with exogenous exposure, so looking at the full list of androgens on page 2 of the DUTCH Complete or page 1 of the Sex Hormone Only panels can help to understand where the high androgens may be coming from.**

**DHEA supplementation can also raise testosterone in women of any age.**

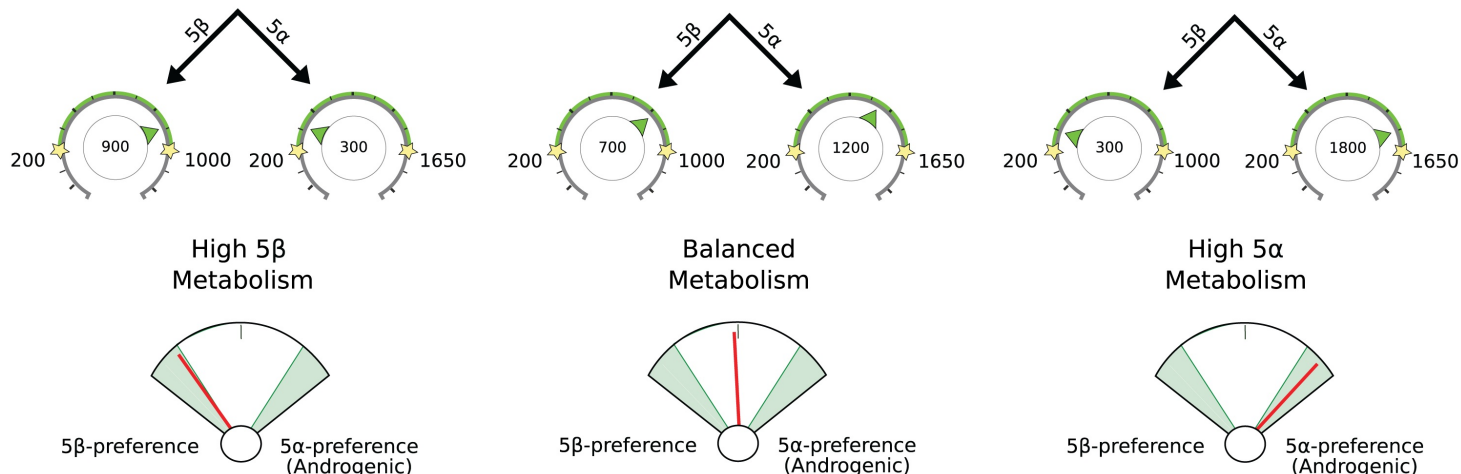
- **Androgen Metabolites: 5 $\alpha$ -reductase versus 5 $\beta$  reductase**

5 $\alpha$ -reductase converts testosterone into 5 $\alpha$ -DHT (DHT), which is even more potent (~3x) than testosterone. High levels of DHT can lead to symptoms associated with too much testosterone, including scalp hair loss, hirsutism, acne and oily skin.

Metabolites created down the 5 $\beta$ -pathway are significantly less androgenic than their 5 $\alpha$  counterparts.

The fan-style gauge below the hormones shows the 5 $\alpha$  or 5 $\beta$  preference based on etiocholanolone (5 $\beta$ ) and androsterone (5 $\alpha$ ) results. The gauge shows the relative ratio of 5 $\alpha$  to 5 $\beta$  products but does not express the absolute value of DHT or if 5 $\alpha$ -reductase inhibition is or is not indicated. Consider symptoms and look at the 5 $\alpha$ -DHT result if high androgen symptoms are a concern. Progesterone metabolites are also metabolized by 5 $\alpha$  and 5 $\beta$  enzymes and the balance between its two metabolites can be useful to confirm a 5 $\alpha$  or 5 $\beta$  preference overall (or tissue specific preference).

Example of how to read fan-style gauge for 5a-reductase activity:



**While the patient has high androgen symptoms, it appears to be more related to higher levels of androgens, including testosterone and not likely related to highly androgenic 5a metabolism.**

When assessing androgens in women, it is important to consider DHEA and testosterone production, 5a-metabolism patterns as well as the patient symptoms. For example, a woman with higher levels of DHEA and testosterone will often have high androgen symptoms (facial hair, thinning scalp hair, etc.) exacerbated by 5a-metabolism.

If, on the other hand, she prefers 5b-metabolism she may not express high androgen symptoms in spite of higher levels of testosterone because 5b is the less androgenic pathway.

You will also see levels of epi-testosterone, which is not androgenic like testosterone. It happens to be produced in about the same concentrations as testosterone (this is an approximate relationship). This can be helpful when assessing the validity of urinary testosterone testing in an individual patient. If epi-testosterone is much higher than testosterone, serum testosterone assessment should be considered before initiating therapy for low testosterone. Epi-testosterone is suppressed when exogenous testosterone is given, which can serve as a proxy for assessing endogenous testosterone production which can be obscured by the exogenous hormone administration.

### DUTCH Adrenal

The HPA-Axis refers to the communication and interaction between the hypothalamus (H) and pituitary (P) in the brain down to the adrenal glands (A) that sit on top of your kidneys. When cortisol is needed in the body, the hypothalamus releases cortisol releasing hormone (CRH) and the pituitary responds by releasing adrenocorticotropic releasing hormone (ACTH), which is the signal to the adrenal gland to release cortisol, DHEA and DHEA-s. It is these adrenal hormones that are assessed on the DUTCH test to understand the patient's HPA axis.

The cortisol awakening response is a complex interaction between the HPA axis and the hippocampus, where ACTH normally surges right after waking leading to the day's highest levels of cortisol. This signal is considered by researchers to be separate from the regular circadian rhythm (the smooth transition from lower cortisol at night to modestly higher cortisol in the morning) and to reflect the person's anticipation of stress during the day, some psychosocial factors such as depression or anxiety and their metabolic state. The waking surge in cortisol helps with energy, focus, morning blood sugar and immune regulation.

As the day progresses, ACTH declines and subsequent cortisol decreases throughout the day, so it is low at night for sleep. This cycle starts over the next morning.

Free cortisol provides negative feedback to CRH & ACTH. When free cortisol is too low, ACTH will surge. ACTH will also surge when a physical or psychological stressor occurs.

Only a small fraction of cortisol is "free" and bioactive. The "free" cortisol is what the person feels in terms of energy and focus. Free cortisol is also what feeds back to the hypothalamus and pituitary gland for ACTH and cortisol regulation. The free cortisol daily pattern is very useful for understanding cortisol and its interaction with the patient's symptoms throughout the day. However, because only a fraction of the cortisol is bioactive, when considering treatments that affect the whole HPA axis, including DHEA, it is essential to measure metabolized

cortisol to get a bigger picture.

In urine, we can measure both the total metabolized cortisol (THF) and total metabolized cortisone (THE) excreted throughout the day. These two components better represent the total cortisol production from the adrenal glands than the free cortisol alone. Outside of the HPA axis, metabolism of cortisol occurs with the help of thyroid hormone in the liver. A significant amount of cortisol is also metabolized in adipose tissue.

To best determine total adrenal production of cortisol throughout the day it is important to assess both metabolized cortisol and free cortisol.

When evaluating cortisol levels, it is important to assess the following:

- **The daily pattern of free cortisol throughout the day, looking for low and high levels:**

Abnormal results should be considered along with related symptoms. Remember that with urine results, the "waking" sample reflects the night's total for free cortisol. The sample collected two hours after waking captures the cortisol awakening response, which is typically the time with the most cortisol secretion.

- **The sum of the free cortisol as an expression of the overall tissue cortisol exposure:**

This total of four free cortisol measurements is the best way to assess the total of free cortisol throughout the day, and this result correlates reasonably well to a true 24-hour urine free cortisol. Do be aware that this measurement does not consider transitory shifts in cortisol in the late morning or early afternoon. This number is calculated from the simple addition of the 4 points, so if a single point is very high or very low, it may skew the number up or down especially if it is the morning "B" point, as it is weighted more heavily in the reference range.

- **The total level of cortisol metabolites:**

We call this calculation "Metabolized Cortisol" which is the sum of a-THF, b-THF and b-THE (the most abundant cortisol metabolites). While free cortisol is the best assessment for tissue levels of cortisol, it only represents 1-3% of the total produced. The total metabolized cortisol best represents the total glandular output of cortisol for the day, closer to 80% of the total produced.

**Free cortisol levels are on the lower side of the reference range. Levels of metabolized cortisol confirm that overall cortisol production is reasonable, and the actual diurnal pattern of free cortisol should be examined to further examine cortisol production.**

- **A potential preference for cortisol or cortisone (the inactive form):**

To determine total systemic preference for cortisol or cortisone, it is best to look at which *metabolite* predominates (THF or THE?). This preference can be seen in the fan style gauge. This is known as the 11b-HSD index. The enzyme 11b-HSD II converts cortisol to cortisone in the kidneys, saliva gland and colon. 11b-HSD I is more active in the liver, fat cells and the periphery and is responsible for reactivating cortisone to cortisol. Both are then metabolized by 5a-reductase to become tetrahydrocortisol (THF) and tetrahydrocortisone (THE) respectively. We can see more cortisol or cortisone in different metabolic conditions. For example, a preference for cortisol indicates possible inflammation, insulin resistance or hypothyroidism. A preference for cortisone can indicate chronic stress or chronic infection (such as the later stages of a virus or common cold).

### **Nutritional Organic Acids**

Organic acids are the metabolic byproducts of cellular activity in the body. Organic acid production varies by the individual and can be influenced by foods, environmental toxins, medications or supplements, nutrient status, genetics and more. Organic acids begin to build up when a nutrient cofactor or mineral is not present for a specific reaction to occur. As a response, byproducts (organic acids) build up and can be measured in urine. On the DUTCH test, the organic acids we measure were chosen due to their specific roles in the metabolism and function of enzymes required for hormone and adrenal health and function. As industry standard dictates, the organic acids are measured from the waking sample.

### **Methylmalonate (MMA)**

Methylmalonic acid is a metabolic byproduct of the Citric Acid Cycle (Krebs cycle). Methylmalonic acid requires adenosylcobalamin for conversion to succinyl-CoA and onto ATP synthesis. If someone does not absorb enough B12 from their diet due to low B12-rich food consumption, low stomach acid, has an autoimmune disorder impacting Intrinsic Factor in the gut (required for B12 absorption), or has an MUT enzyme SNP (required for conversion of MMA to Succinyl coA, dependent on adenosylcobalamin) then MMA will build up. Vitamin B12 is required for COMT activity (estrogen methylation, dopamine breakdown) and PNMT activity (the enzyme that takes norepinephrine to epinephrine), but is also critical for memory, energy production (ATP synthesis), gait and more. When MMA is high, consider supporting B12 through foods, digestive support or supplementation.

### **Xanthurenate & Kynurenate**



Xanthurenate and kynurenate are metabolic byproducts in the production of tryptophan to NAD in the liver. If either xanthurenate or kynurenate build up in the urine, it can indicate a need for vitamin B6. This need is amplified if BOTH markers are elevated, and often indicates a more severe deficiency of vitamin B6. Vitamin B6 is critical as a co-factor to over 100 important reactions that occur in the human body and is stored in the highest concentration in muscle tissue.

Tryptophan is converted to NAD by the liver and one of the steps in this pathway requires B6. When B6 is insufficient, xanthurenate is made instead. Xanthurenate can also bind to iron and create a complex that increases DNA oxidative damage resulting in higher 8-OHdG levels. If both the xanthurenate and 8OHdG levels are elevated, there is likely an antioxidant insufficiency.

Kynurenate may also become elevated when patients are B6 deficient because of a different, possibly less B6 dependent pathway. While there is always some tryptophan going down the kynurenine pathway towards NAD, and possibly xanthurenate, this process is up regulated by inflammation, estrogen and cortisol elevations. If levels of estrogen or cortisol are high, it may exacerbate kynurenic acid and increase the need for vitamin B6. As the Xanthurenate and Kynurenate pathways lead to biomarkers with other influence in the body, elevations in these markers may not always agree.

### **Pyroglutamate**

Pyroglutamate is an intermediate in glutathione recycling and production. Glutathione requires the amino acids cysteine, glycine and glutamate for production. If the body cannot convert pyroglutamate forward to glutathione, it will show up elevated in the urine. High pyroglutamate is an established marker for glutathione deficiency.

Remember that glutathione is one of the most potent antioxidants in the human body and is especially important in getting rid of toxins including the reactive quinone species formed by 4-OH-E1 and 4-OH-E2. This reactive species can damage DNA if not detoxified by either methylation or glutathione.

Some have reported that low pyroglutamate may also be indicative of a need for glutathione; however, this is not established in the scientific literature.

*Note: Pyroglutamate in the urine can also be elevated with Italian cheese consumption. Italian Cheeses (parmesan, etc.) may transiently increase pyroglutamate because they use a thermophilic lactobacilli to ripen the cheese- which our gut breaks down into pyroglutamate. This is not clinically significant and only reflects that they ate this style of cheese (if applicable).*

### **Neurotransmitter Metabolites**

Neurotransmitters are chemical signals produced by neurons in tissues throughout the body that act as chemical messengers that influence mood, cortisol, heart rate, appetite, muscle contraction, sleep and more. Measuring neurotransmitters directly is difficult because of their instability, and their direct urinary measurements are controversial with respect to how well they reflect the body's level of these neuro-hormones.

Each of the neurotransmitters assessed on the DUTCH test (dopamine, norepinephrine/epinephrine) can be assessed indirectly by measuring their urine metabolites (HVA and VMA respectively). While these metabolites are not a perfect reflection of what is going on in the brain, the scientific literature does affirm their use for a good representation of overall levels of these neurotransmitters in the body.

### **Homovanillate (HVA)**

Homovanillate (HVA) is the primary metabolite of dopamine, a brain and adrenal neurotransmitter that comes from tyrosine (with BH4 and iron as co-factors). Dopamine goes on to create norepinephrine and epinephrine (adrenaline).

Low levels of dopamine are associated with depression, addictions, cravings, apathy, pleasure seeking behaviors, increased sleepiness, impulsivity, tremors, low motivation fatigue and low mood.

High levels of dopamine are associated with agitation, insomnia, mania, hyperactivity, hyper-focus, high stress, anxiety and addictions/cravings/pleasure seeking (to maintain high levels).

High HVA can be caused by the use of the following supplements, foods or medications within 72 hours of collecting urine samples: tyrosine, phenylalanine, mucuna, quercetin, bananas, avocados as well as parkinson's medications. If these are being used, the HVA on the DUTCH test may not accurately reflect circulating dopamine levels and should be disregarded.

### **Vanilmandelate (VMA)**

Vanilmandelate (VMA) is the primary metabolite of norepinephrine and epinephrine (adrenaline). The adrenal gland makes cortisol and DHEA (from the adrenal cortex) as well as norepinephrine and epinephrine (from the adrenal medulla). When adrenal hormone output is low, VMA levels may be low. If HVA levels are significantly higher than VMA, there may be a conversion problem from dopamine to norepinephrine. This case can be

caused by a copper or vitamin C deficiency.

The enzymes COMT (methylation of catechols) and MAO are needed to make HVA and VMA from dopamine and norepinephrine respectively. If these enzymes are not working properly, HVA and/or VMA may be low in urine, when circulating levels of dopamine and/or norepinephrine/epinephrine may not be low.

Low levels of norepinephrine/epinephrine are associated with addictions, cravings, fatigue, low blood pressure, low muscle tone, intolerance to exercise, depression, and loss of alertness.

High levels of norepinephrine and epinephrine are associated with feelings of stress, aggression, violence, impatience, anxiety, panic, excess worry/hypervigilance, insomnia, paranoia, increasing tingling/burning, loss of memory, pain sensitivity, high blood pressure and heart palpitations.

### **Melatonin (measured as 6-OHMS)**

Melatonin is considered one of our sleep hormones. It is made predominately by the pineal gland in response to darkness and is stimulated by melanocyte stimulating hormone (MSH). A low MSH is associated with insomnia and an increased perception of pain. Mold exposure can inhibit MSH as well. The majority of our melatonin production comes from the pineal gland, but melatonin is also made in the gut, and to a lesser extent in the bone marrow, lymphocytes, epithelial cells and mast cells.

Please note that some foods contain small amounts of melatonin that are unlikely to increase circulating levels of melatonin, but may increase metabolites in urine due to first pass metabolism. The most significant of these foods are tomatoes, walnuts, strawberries and caffeinated coffee. These foods are thought to contribute to mildly elevated urinary melatonin. Extremely high urinary melatonin is seen when melatonin is supplemented directly. This is also due to first pass metabolism and is not an accurate reflection of circulating melatonin.

*The DUTCH test uses the waking (A) sample to test melatonin. The urine sample given on waking reflects overnight hormone production and metabolism. This sample can be used to assess melatonin throughout the night. When patients take a middle of the night urine sample, a large amount of data strongly suggests that the waking sample alone still correlates best to overnight melatonin production, so the waking sample is still used for the DUTCH melatonin result.*

### **8-OHdG (8-Hydroxy-2-deoxyguanosine)**

8-OHdG (8-Hydroxy-2-deoxyguanosine) is a marker for estimating DNA damage due to oxidative stress (from ROS creation). 8-OHdG is considered pro-mutagenic and is a biomarker for various cancer and degenerative disease initiation and promotion states. It can be increased by chronic inflammation, increased cell turnover, chronic stress, hypertension, hyperglycemia/pre-diabetes/diabetes, kidney disease, IBD, chronic skin conditions (psoriasis/eczema), depression, atherosclerosis, chronic liver disease, Parkinson's (increasing levels with worsening stages), Diabetic neuropathy, COPD, bladder cancer, or insomnia (to name a few). Studies have shown higher levels in patients with breast and prostate cancers. When levels are elevated it may be prudent to eliminate or reduce any causes and increase the consumption of antioxidant containing foods and/or supplements.

### **Urine Hormone Testing - General Information**

What is actually measured in urine? In blood, most hormones are bound to binding proteins. A small fraction of the total hormone levels are "free" and unbound such that they are active hormones. These free hormones are not found readily in urine except for cortisol and cortisone (because they are much more water soluble than, for example, testosterone). As such, free cortisol and cortisone can be measured in urine and it is this measurement that nearly all urinary cortisol research is based upon. In the DUTCH Adrenal Profile the diurnal patterns of free cortisol and cortisone are measured by LC-MS/MS.

All other hormones measured (cortisol metabolites, DHEA, and all sex hormones) are excreted in urine predominately after the addition of a glucuronide or sulfate group (to increase water solubility for excretion). As an example, Tajic (Natural Sciences, 1968 publication) found that of the testosterone found in urine, 57-80% was testosterone-glucuronide, 14-42% was testosterone-sulfate, and negligible amounts (<1% for most) was free testosterone. The most likely source of free sex hormones in urine is from contamination from hormonal supplements. To eliminate this potential, we remove free hormones from conjugates (our testing can be used even if vaginal hormones have been given). The glucuronides and sulfates are then broken off of the parent hormones, and the measurement is made. These measurements reflect the bioavailable amount of hormone in most cases as it is only the free, nonprotein-bound fraction in blood/tissue that is available for phase II metabolism (glucuronidation and sulfation) and subsequent urine excretion.

Disclaimer: the filter paper used for sample collection is designed for blood collection, so it is technically considered "research only" for urine collection. Its proper use for urine collection has been thoroughly validated.



## Reference Range Determination (last updated 11.23.2021)

We aim to make the reference ranges for our DUTCH tests as clinically appropriate and useful as possible. This includes the testing of thousands of healthy individuals and combing through the data to exclude those that are not considered "healthy" or "normal" with respect to a particular hormone. As an example, we only use a premenopausal woman's data for estrogen range determination if the associated progesterone result is within the luteal range (days 19-21 when progesterone should be at its peak). We exclude women on birth control or with any conditions that may be related to estrogen production. Over time the database of results for reference ranges has grown quite large. This has allowed us to refine some of the ranges to optimize for clinical utility. The manner in which a metabolite's range is determined can be different depending on the nature of the metabolite. For example, it would not make clinical sense to tell a patient they are deficient in the carcinogenic estrogen metabolite, 4-OH-E1 therefore the lower range limit for this metabolite is set to zero for both men and women. Modestly elevated testosterone is associated with unwanted symptoms in women more so than in men, so the high range limit is set at the 80th percentile in women and the 90th percentile for men. Note: the 90th percentile is defined as a result higher than 90% (9 out of 10) of a healthy population.

Classic reference ranges for disease determination are usually calculated by determining the average value and adding and subtracting two standard deviations from the average, which defines 95% of the population as being "normal." When testing cortisol, for example, these types of two standard deviation ranges are effective for determining if a patient might have Addison's (very low cortisol) or Cushing's (very high cortisol) Disease. Our ranges are set more tightly to be optimally used for Functional Medicine practices.

Below you will find a description of the range for each test:

Female Reference Ranges (Updated 11.23.2021)									
	Low%	High%	Low	High		Low%	High%	Low	High
b-Pregnanediol	20%	90%	600	2000	Cortisol A (waking)	20%	90%	10	50
a-Pregnanediol	20%	90%	200	740	Cortisol B (morning)	20%	90%	30	130
Estrone (E1)	20%	80%	12	26	Cortisol C (~5pm)	20%	90%	7	30
Estradiol (E2)	20%	80%	1.8	4.5	Cortisol D (bed)	0	90%	0	14
Estriol (E3)	20%	80%	5	18	Cortisone A (waking)	20%	90%	40	120
2-OH-E1	20%	80%	5.1	13.1	Cortisone B (morning)	20%	90%	90	230
4-OH-E1	0	80%	0	1.8	Cortisone C (~5pm)	20%	90%	32	110
16-OH-E1	20%	80%	0.7	2.6	Cortisone D (bed)	0	90%	0	55
2-Methoxy-E1	20%	80%	2.5	6.5	Melatonin (6-OHMS)	20%	90%	10	85
2-OH-E2	0	80%	0	1.2	8-OHdG	0	90%	0	5.2
4-OH-E2	0	80%	0	0.5	Methylmalonate	0	90%	0	2.2
DHEA-S	20%	90%	20	750	Xanthurenate	0	90%	0	1.4
Androsterone	20%	80%	200	1650	Kynurenate	0	90%	0	7.3
Etiocholanolone	20%	80%	200	1000	Pyroglutamate	10%	90%	32	60
Testosterone	20%	80%	2.3	14	Homovanillate	10%	95%	4	13
5a-DHT	0	80%	0	6.6	Vanilmandelate	10%	95%	2.4	6.4
5a-Androstenediol	20%	80%	6	30					
5b-Androstenediol	20%	80%	20	75	<b>Calculated Values</b>				
Epi-Testosterone	20%	80%	2.3	14	Total DHEA Production	20%	80%	500	3000
a-THF	20%	90%	75	370	Total Estrogens	20%	80%	35	70
b-THF	20%	90%	1050	2500	Metabolized Cortisol	20%	90%	2750	6500
b-THE	20%	90%	1550	3800	24hr Free Cortisol	20%	90%	65	200
					24hr Free Cortisone	20%	90%	220	450
% = population percentile: Example - a high limit of 90% means results higher than 90% of the women tested for the reference range will be designated as "high."									

### Provider Notes:

# Normal or Short Cycle

SAMPLE	CYCLE	DATE
#1	Day 7	6/12/21
#2	Day 9	8/12/21
#3	Day 10	9/12/21
#4 <b>(1)</b>	Day 11	10/12/21
#5	Day 12	11/12/21
#6 <b>(2)</b>	Day 13	12/12/21
#7 <b>(3)</b>	Day 14	13/12/21
#8 <b>(4)</b>	Day 15	14/12/21
#9	Day 16	15/12/21
#10 <b>(5)</b>	Day 17	16/12/21
#11	Day 18	17/12/21
#12	Day 19	18/12/21
#13	Day 20	19/12/21
#14 <b>(6)</b>	Day 21	20/12/21
#15	Day 22	21/12/21

SAMPLE	CYCLE	DATE
#16	Day 24	23/12/21
#17 <b>(7)</b>	Day 26	25/12/21
#18	Day 28	Jan Lost
#19 <b>(8)</b>	Day 30	29/12/21
#20	Day 33	
#21	Day 36	

New cycle  
start date:  
(1st day of menstrual  
flow)

12/29

## Last 4 Samples

SAMPLE	TIME	DATE
#22 <b>A</b>	7:20	1/1/22
#23 <b>B</b>	9:20	1/1/22
#24 <b>C</b>	17:20	1/1/22
#25 <b>D</b>	22:20	1/1/22

Ceto