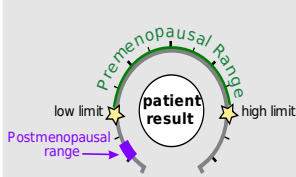


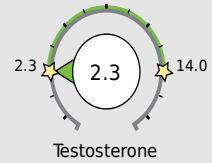
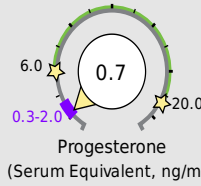
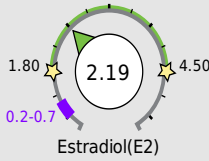
TEST NAME: DUTCH Plus™

Hormone Testing Summary

Key (how to read the results):

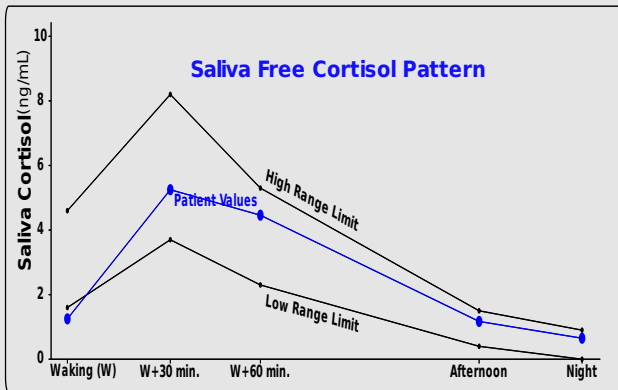


Sex Hormones See Pages 2 and 3 for a thorough breakdown of sex hormone metabolites



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



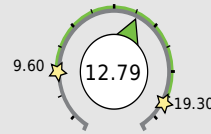
Free cortisol best reflects tissue levels. Metabolized cortisol best reflects total cortisol production.

Total DHEA Production

Age	Range
20-39	1300-3000
40-59	750-2000
>60	500-1200

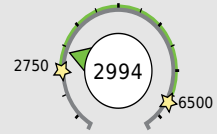


Total DHEA Production (DHEAS + Etiocholanolone + Androsterone)



Saliva Cortisol Total (Sum of 5 values)

cortisol metabolism



Metabolized Cortisol (THF+THE) (Total Cortisol Production)

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

The Cortisol Awakening Response (CAR) was 4.00ng/mL (expected range 1.5-4.0) or 320% (range 50-160%). See page 5 for more details.



PATIENT: XXXXXXXXXXXXXXXX

TEST NUMBER: G-NL-XXXXX

GENDER: XXXXXX

AGE: XX

COLLECTED: 00-XXX-2024

RECEIVED: 00-XXX-2024

TESTED: 00-XXX-2024

TEST REF: GNL-NL-XXXXX

PRACTITIONER:
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TEST NAME: DUTCH Plus™

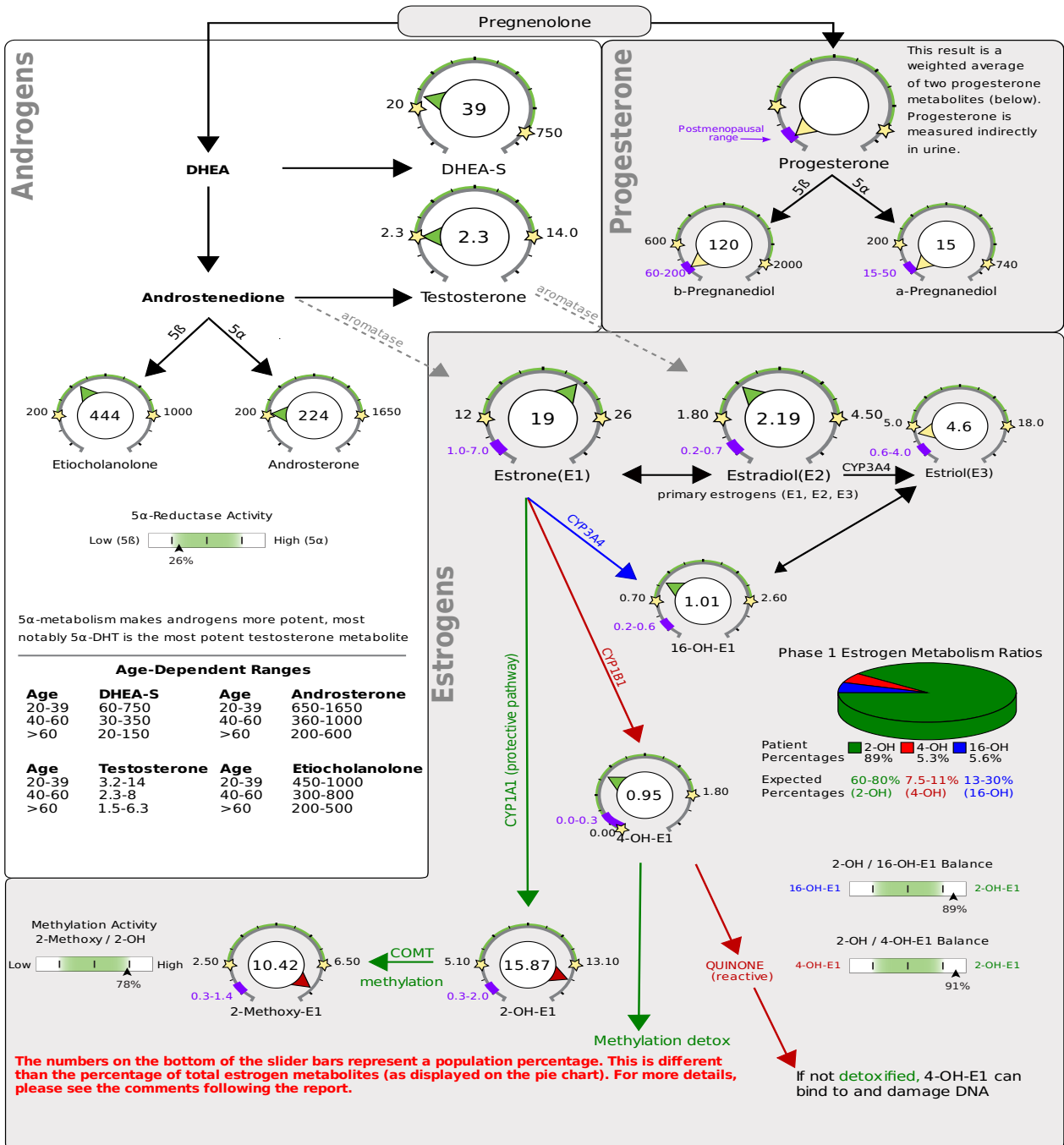
Test		Result	Units	Luteal*	Postmenopausal
Progesterone Metabolites (Urine)					
b-Pregnanediol	Below luteal range	120.3	ng/mg	600 - 2000	60-200
a-Pregnanediol	Below luteal range	15.4	ng/mg	200 - 740	15-50
Estrogens and Metabolites (Urine)					
Estrone(E1)	Within luteal range	19.08	ng/mg	12 - 26	1.0-7.0
Estradiol(E2)	Low end of luteal range	2.19	ng/mg	1.8 - 4.5	0.2-0.7
Estriol(E3)	Below luteal range	4.6	ng/mg	5 - 18	0.6-4.0
2-OH-E1	Above luteal range	15.87	ng/mg	5.1 - 13.1	0.3-2.0
4-OH-E1	Within luteal range	0.95	ng/mg	0 - 1.8	0-0.3
16-OH-E1	Low end of luteal range	1.01	ng/mg	0.7 - 2.6	0.2-0.6
2-Methoxy-E1	Above luteal range	10.42	ng/mg	2.5 - 6.5	0.3-1.4
2-OH-E2	Above luteal range	1.28	ng/mg	0 - 1.2	0-0.3
4-OH-E2	High end of luteal range	0.42	ng/mg	0 - 0.5	0-0.1
Total Estrogen	Within range	55.8	ng/mg	35 - 70	4.0-15
Metabolite Ratios					
2-OH / 16-OH-E1 Balance	Above range	15.77	ratio	2.69 - 11.83	
2-OH / 4-OH-E1 Balance	Above range	16.69	ratio	5.4 - 12.62	
2-Methoxy / 2-OH Balance	High end of range	0.66	ratio	0.39 - 0.67	
Androgens and Metabolites (Urine)					
DHEA-S	Low end of range	38.7	ng/mg	20 - 750	
Androsterone	Low end of range	224.4	ng/mg	200 - 1650	
Etiocholanolone	Within range	443.9	ng/mg	200 - 1000	
Testosterone	Low end of range	2.3	ng/mg	2.3 - 14	
5a-DHT	Within range	0.8	ng/mg	0 - 6.6	
5a-Androstanediol	Below range	3.9	ng/mg	6 - 30	
5b-Androstanediol	Below range	15.0	ng/mg	20 - 75	
Epi-Testosterone	Below range	0.9	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

TEST NAME: DUTCH Plus™

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.





PATIENT: XXXXXXXXXXXXXXXX

TEST NUMBER: G-NL-XXXXX

GENDER: XXXXXX

AGE: XX

COLLECTED: 00-XXX-2024

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TESTED: 00-XXX-2024

TEST REF: GNL-NL-XXXXX

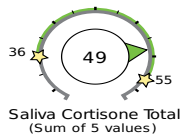
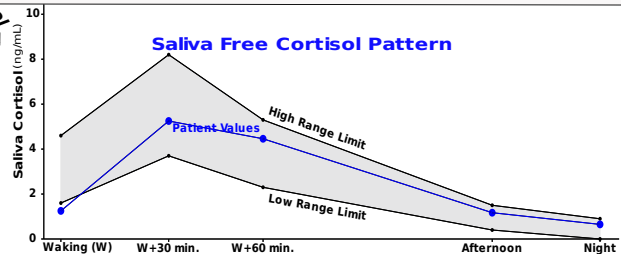
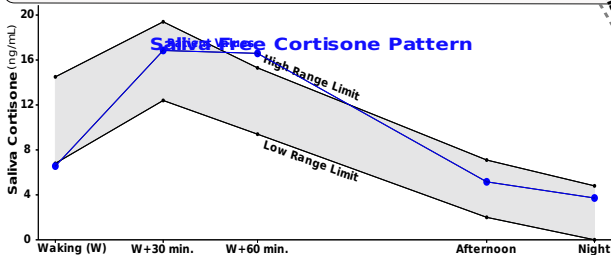
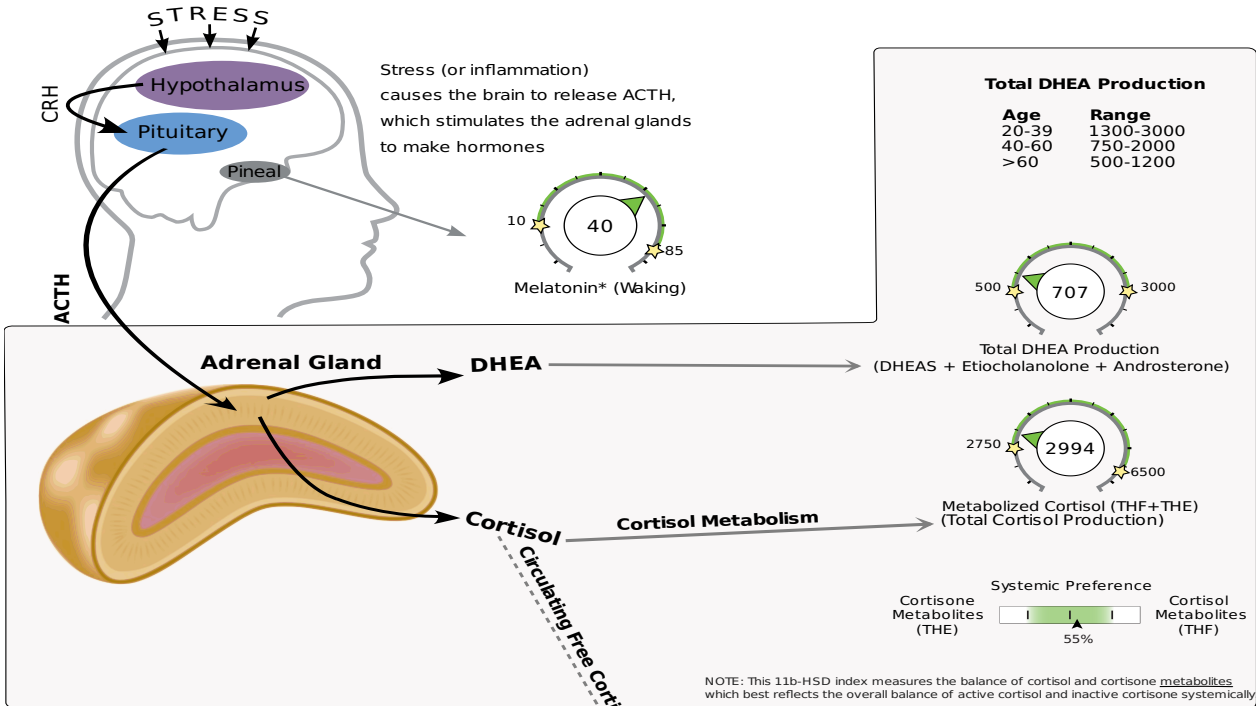
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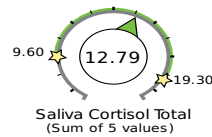
TEST NAME: DUTCH Plus™

Category	Test	Result	Units	Normal Range
Free Cortisol and Cortisone (Saliva)				
	Saliva Cortisol - Waking (W)	Below range	1.25	ng/mL 1.6 - 4.6
	Saliva Cortisol - W+30 min.	Within range	5.25	ng/mL 3.7 - 8.2
	Saliva Cortisol - W+60 min.	Within range	4.46	ng/mL 2.3 - 5.3
	Saliva Cortisol - Afternoon	Within range	1.17	ng/mL 0.4 - 1.5
	Saliva Cortisol - Night	Within range	0.65	ng/mL 0 - 0.9
	Saliva Cortisone - Waking (W)	Below range	6.56	ng/mL 6.8 - 14.5
	Saliva Cortisone - W+30 min.	Within range	16.85	ng/mL 12.4 - 19.4
	Saliva Cortisone - W+60 min.	Above range	16.59	ng/mL 9.4 - 15.3
	Saliva Cortisone - Afternoon	Within range	5.16	ng/mL 2 - 7.1
	Saliva Cortisone - Night	Within range	3.72	ng/mL 0 - 4.8
	Saliva Cortisol Total	Within range	12.79	ng/mL 9.6 - 19.3
	Saliva Cortisone Total	Within range	48.87	ng/mL 36 - 55
Creatinine (Urine)				
	Creatinine A (Waking)	Within range	0.62	mg/ml 0.2 - 2
	Creatinine B (Morning)	Within range	0.74	mg/ml 0.2 - 2
	Creatinine C (Afternoon)	Within range	0.64	mg/ml 0.2 - 2
	Creatinine D (Night)	Within range	1.04	mg/ml 0.2 - 2
Cortisol Metabolites and DHEA-S (Urine)				
	a-Tetrahydrocortisol (a-THF)	Low end of range	85.2	ng/mg 75 - 370
	b-Tetrahydrocortisol (b-THF)	Low end of range	1241.6	ng/mg 1050 - 2500
	b-Tetrahydrocortisone (b-THE)	Low end of range	1667.1	ng/mg 1550 - 3800
	Metabolized Cortisol (THF+THE)	Low end of range	2993.8	ng/mg 2750 - 6500
	DHEA-S	Low end of range	38.7	ng/mg 20 - 750

TEST NAME: DUTCH Plus™



Cortisol and Cortisone interconvert (11b-HSD)



The Cortisol Awakening Response (CAR) is the rise in salivary cortisol between the waking sample and the sample collected 30 (as well as 60) minutes later. This "awakening response" is essentially a "mini stress test" and is a useful measurement in addition to the overall up-and-down (diurnal) pattern of free cortisol throughout the day. **This patient shows a waking cortisol of 1.25 and an increase to 5.2 after 34.0 minutes. This is an increase of 4.00ng/mL or 320%.** Expected increases differ depending on the methods used. Preliminary research shows that 50-160% or 1.5-4.0ng/mL increases are common with samples collected 30 minutes after waking. These guidelines are considered research only. **This patient shows a salivary cortisol of 4.46 measured 60 minutes after waking. This is an increase of 3.21ng/mL or 257% compared to the waking sample.** To date, data suggests that expected results may be 0-70%, and this guideline is considered for research only.



PATIENT: XXXXXXXXXXXXXXXX

TEST NUMBER: G-NL-XXXXXX

GENDER: XXXXXXX

AGE: XX

COLLECTED: 00-XXX-2024

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TESTED: 00-XXX-2024

TEST REF: GNL-NL-XXXXX

PRACTITIONER:
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TEST NAME: DUTCH Plus™

Category	Test	Result	Units	Normal Range
Nutritional Organic Acids				
Vitamin B12 Marker (may be deficient if high) - (Urine)				
	Methylmalonate (MMA)	Within range	1.5 ug/mg	0 - 2.5
Vitamin B6 Markers (may be deficient if high) - (Urine)				
	Xanthurenate	Within range	0.77 ug/mg	0.12 - 1.2
	Kynurenate	Within range	2.9 ug/mg	0.8 - 4.5
Biotin Marker (may be deficient if high) - (Urine)				
	b-Hydroxyisovalerate	Within range	6.1 ug/mg	0 - 12.5
Glutathione Marker (may be deficient if low or high) - (Urine)				
	Pyroglutamate	Within range	41.9 ug/mg	28 - 58
Gut Marker (potential gut putrefaction or dysbiosis if high) - (Urine)				
	Indican	Within range	35.4 ug/mg	0 - 100
Neuro-related Markers				
Dopamine Metabolite - (Urine)				
	Homovanillate (HVA)	Within range	3.8 ug/mg	3 - 11
Norepinephrine/Epinephrine Metabolite - (Urine)				
	Vanilmandelate (VMA)	Low end of range	2.2 ug/mg	2.2 - 5.5
Neuroinflammation Marker - (Urine)				
	Quinolinat	Within range	3.5 ug/mg	0 - 9.6
Additional Markers				
Melatonin (*measured as 6-OH-Melatonin-Sulfate) - (Urine)				
	Melatonin* (Waking)	Within range	39.9 ng/mg	10 - 85
Oxidative Stress / DNA Damage, measured as 8-Hydroxy-2-deoxyguanosine (8-OHdG) - (Urine)				
	8-OHdG (Waking)	Within range	1.7 ng/mg	0 - 5.2



PATIENT: XXXXXXXXXXXXXXXX

TEST NUMBER: G-NL-XXXXX

GENDER: XXXXXX

AGE: XX

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TESTED: 00-XXX-2024

TEST REF: GNL-NL-XXXXX

PRACTITIONER:

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TEST NAME: DUTCH Plus™

Clinical Support Overview

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Page: 7 of 19

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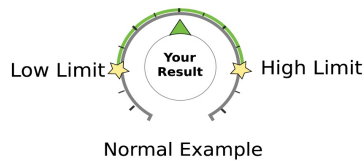
TEST NAME: DUTCH Plus™

How to read the DUTCH report

This report is not intended to treat, cure or diagnose any specific diseases.

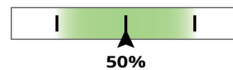
DUTCH Dials

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.



DUTCH Slider Bars

In a few places on the graphic pages, you will see slider bars. For adrenal hormones, you will see one to represent the balance between cortisol and cortisone metabolites. These bars indicate the relative ratio of the metabolites noted. The percentage stated is a population percentage, and so a result of 50%, as in this example (with the slider arrow in the middle of the bar) indicates that the ratio is higher than 50% of individuals tested, or right in the middle of the population's range. If the ratio between the metabolites is "low", the arrow will slide to the left and represent a smaller percentage 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 no menstrual cycles.

The patient reports symptoms of both estrogen deficiency and excess. Levels of estrogen (and metabolites) should be carefully reviewed.

The patient reported significant symptoms of excess androgen levels.

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



PATIENT: XXXXXXXXXXXXXXXX

TEST NUMBER: G-NL-XXXXX

GENDER: XXXXXX

AGE: XX

COLLECTED: 00-XXX-2024

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TEST REF: GNL-NL-XXXXX

PRACTITIONER:
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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.

The weighted average of the two progesterone metabolites shows progesterone is in range for post menopause. Postmenopausal progesterone is produced by the adrenal glands. Progesterone supplementation may be appropriate for improving energy, sleep and mood, even when the patient's levels are normal for menopause.

Estrogen Metabolism

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

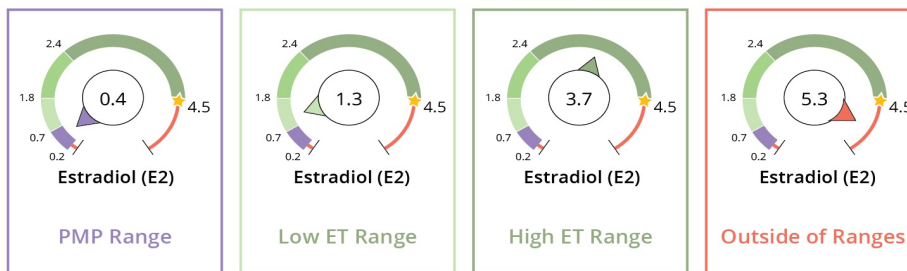
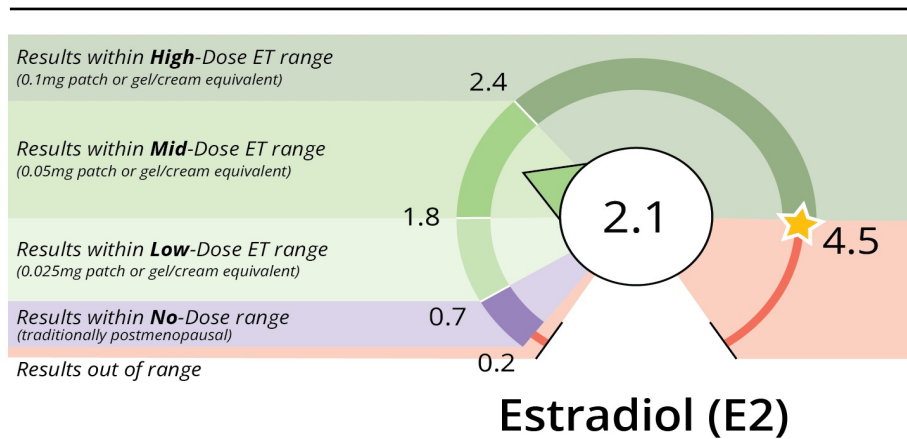
• **The status (low, normal or high?) of estrogen production:**

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).

The patient reports the use of transdermal estrogen replacement therapy (ERT). Urine levels of estradiol (E2) have been shown to generally parallel serum levels with transdermal ERT. Precision Analytical does not recommend any one-size-fits-all ideal range for all patients on transdermal E2 because each patient will have different responses to therapy and different goals. Our research results can help providers and patients make informed decisions about ERT.

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Estradiol Dial Modified to Include Observed Ranges for Females on Transdermal (TD) Estrogen Therapy (ET)



This study, peer-reviewed by the rigorous team at Menopause (Journal of The North American Menopause Society (NAMS)), highlights the utility of urine metabolite testing to assist in the evaluation of transdermal ERT. Patients on low to moderate doses of transdermal E2 gels or creams show DUTCH test E2 levels between 0.7-2.4 ng/mg.

A patient whose E2 levels remain below 0.7 ng/mg (postmenopausal range) may not have the same level of clinical benefit as a patient with a higher E2 concentration. Individual absorption and symptom improvement vary from person to person. When transdermal E2 is applied daily, levels vary over the day. DUTCH testing shows an average hormone level over the 24-hour period that samples are collected. This makes testing helpful for monitoring ERT. DUTCH also measures estrogen metabolism which may improve long-term clinical outcomes. Precision Analytical does not endorse any dose or type of therapy for ERT.

• **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



PATIENT: XXXXXXXXXXXXXXXX

TEST NUMBER: G-NL-XXXXX

GENDER: XXXXXX

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TEST REF: GNL-NL-XXXXX

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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.

• 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

Androgen Metabolites: DHEA

DHEA and androstenedione are made almost exclusively by the adrenal glands. 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. Adrenals serve as the main source of estrogen, progesterone and testosterone for post-menopausal women.

• 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 (5a-androstanediol, 5bandrostanediol). 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

TEST NAME: DUTCH Plus™

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 result 2.30ng/mg is in range for the patient's age. Review the levels of all androgens, androgenic metabolism, and patient symptoms for a complete assessment.

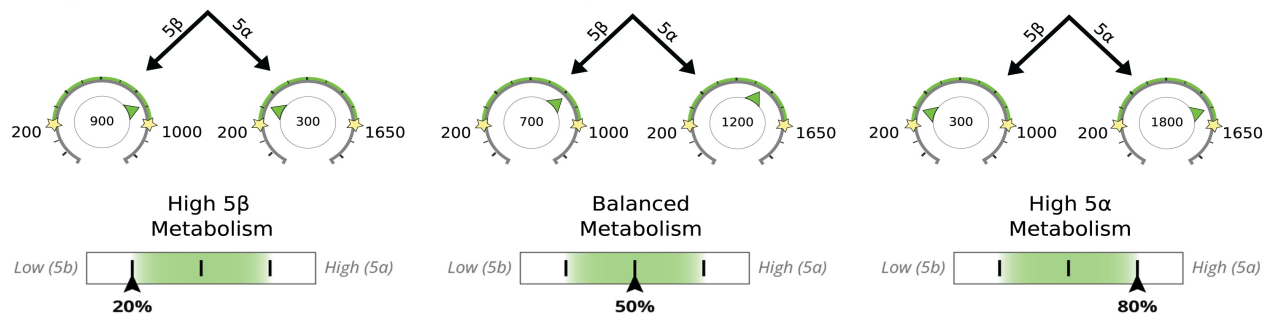
• **Androgen Metabolites: 5a-reductase versus 5b reductase**

5a-reductase converts testosterone into 5a-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 5b-pathway are significantly less androgenic than their 5a counterparts.

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

Example of how to read sliders for 5a-reductase activity:



Neither testosterone or overall levels of DHEA are elevated, and 5a-metabolism is not elevated. However, the patient does complain of some high androgen symptoms. If the primary complaint is thinning scalp hair, it is not likely that DHT production is to blame. Other causes of these symptoms (thyroid, cortisol, iron...etc) should be explored.

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 initiated 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



PATIENT: XXXXXXXXXXXXXXXX

TEST NUMBER: G-NL-XXXXX

GENDER: XXXXXX

AGE: XX

COLLECTED: 00-XXX-2024

RECEIVED: 00-XXX-2024

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TEST REF: GNL-NL-XXXXX

PRACTITIONER:
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TEST NAME: DUTCH Plus™

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, and it 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.

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 measure 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**

The patient is instructed to collect on a "typical" day because cortisol, as an acute response hormone, can vary from day to day if activities are very different. Abnormal results should be considered along with the patient's symptoms and any unusual occurrences of the day.

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

This total of five free cortisol measurements is the best way to assess the total of free cortisol throughout the day, but do be aware that it is heavily weighted towards the morning production since three of five measurements are made within the first hour of the day.

- **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 for the day.

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

Looking at the comparison between the total for free cortisol and free cortisone is NOT the best indication of a person's preference for cortisol or cortisone. The saliva gland converts cortisol to cortisone in the local tissue. This localized conversion can be seen by comparing cortisol (free) and cortisone levels. To know how much free cortisol was made by the adrenals we must know how much was deactivated to free cortisone at the level of the saliva gland. **However, to determine total systemic preference of steroid activity, it is best to look at which metabolite predominates (THF or THE?).** This preference can be seen in the slider bar. 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.

- **The Cortisol Awakening Response (CAR):**

The unique feature of the DUTCH Plus is the inclusion of the CAR assessment. The response to waking adds one more piece to HPA-axis function. In some cases, overall levels of free cortisol may be normal, but the response to stress may be under or overactive.

The Cortisol Awakening Response is measured as a percent difference between the waking and 30-minute (peak)



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cortisol. Additional information can be gathered by further measuring the percent difference between the waking and 60-minute (recovery) cortisol. This up and down pattern is thought to reflect the individual's natural response to stress, where the act of waking up serves as a mini "stress test". In addition to the CAR, the overall total can be assessed by looking at the salivary cortisol total as well as the individual points.

Reasons for a lower CAR might include: an underactive HPA Axis, excessive psychological burnout, seasonal affective disorder (SAD), sleep apnea or poor sleep in general, PTSD, and "chronic fatigue" patients.

An elevated CAR can be a result of an over-reactive HPA axis, ongoing job-related stress (anticipatory stress for the day), glycemic dysregulation, pain (ie. waking with painful joints or a migraine), and general depression (not SAD). Scientific literature points to the magnitude of the morning cortisol increase as being connected to HPA-axis health whether the overall production of cortisol is low, normal or high.

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 a 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 kynurenine 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.

b-Hydroxyisovalerate

b-Hydroxyisovalerate is made when the body is deficient in biotin. Elevated levels may indicate biotin deficiency. Biotin is an important cofactor in mitochondrial function, metabolism of fatty acids, glucose, and protein, and ROS production. Biotin deficiency has similar symptoms as other B-vitamin deficiencies but is most often associated with hair loss. Factors influencing biotin levels include inadequate dietary intake, long-term and high-dose B5 supplementation, dysbiosis/gut health, antibiotic use, medications, and biotinidase deficiency. Note: If beta-hydroxy-beta-methylbutyrate (HMB), an amino acid conjugate supplement, is taken within 72 hours of testing, we can see high levels of b-hydroxyisovalerate without indicating a biotin deficiency. Please check with supplements when interpreting results.

Pyroglutamate

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PATIENT: XXXXXXXXXXXXXXXX

TEST NUMBER: G-NL-XXXXXX

GENDER: XXXXXX

AGE: XX

COLLECTED: 00-XXX-2024

RECEIVED: 00-XXX-2024

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TEST REF: GNL-NL-XXXXX

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TEST NAME: DUTCH Plus™

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).

Indican

Indican is a byproduct of tryptophan putrefaction by microbes in the gut. Accumulated levels of indican in the urine suggest higher levels of tryptophan putrefaction from gastrointestinal dysbiosis or malabsorption. Production of indican occurs when tryptophan creates indoles in the colon. No other endogenous indoles are metabolized in this way, so when we see indican in the urine, it is directly related to gut production and a direct reflection of gut health. Chronic dysbiosis can impact sex hormone metabolism, cause inflammation, and influence cortisol levels and metabolism. High urinary indican suggests further testing to rule out gut dysbiosis.

Vegetarian and vegan diets have less protein, therefore elevated levels with these diets are likely stronger suggestions of gut dysbiosis. The amount of indican present does not correlate to the degree of dysbiosis but merely shows that dysbiosis is present. Common causes of high indican include malabsorption of protein because of low stomach acid, poor pancreatic function, Celiac disease, the overgrowth of anaerobic bacteria in the colon, small intestinal bacterial overgrowth (SIBO), medications that reduce protein absorption (like proton pump inhibitors or other antacids or H2 blockers), and constipation. Urinary indican can increase with recent (<72 hours) tryptophan supplementation without indicating dysbiosis. Please keep supplements in mind when interpreting the result.

Neuro-related Markers

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.



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PRACTITIONER:

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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.

Quinolate (QA)

The quinolate is high. Quinolate is a neurotoxin derived from tryptophan metabolism in the context of high inflammation or high cortisol. Elevated quinolate has been seen in brain and nerve tissue damage, especially in disorders such as Alzheimer's disease, Parkinson's disease, Huntington's disease, motor neuron diseases, multiple sclerosis, epilepsy, amyotrophic lateral sclerosis, and major depressive disorder. We can also see elevated quinolate due to low serotonin and need for vitamin B3 (niacin). If tryptophan supplements are taken within 72 hours of collecting DUTCH samples, there may be high levels of quinolate in the urine which may not be associated with neuroinflammation. Keep supplements in mind when interpreting results.

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.



PATIENT: XXXXXXXXXXXXXXXX

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GENDER: XXXXXX

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TEST REF: GNL-NL-XXXXX

PRACTITIONER:
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TEST NAME: DUTCH Plus™

Reference Range Determination (last updated 05.1.2024)

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 05.01.2024)									
	Low%	High%	Low	High		Low%	High%	Low	High
b-Pregnanediol	20%	90%	600	2000	Saliva Cortisol Waking (W)	20%	90%	1.6	4.6
a-Pregnanediol	20%	90%	200	740	Saliva Cortisol (W+30 min.)	20%	90%	3.7	8.2
Estrone (E1)	20%	80%	12	26	Saliva Cortisol (W+60 min.)	20%	90%	2.3	5.3
Estradiol (E2)	20%	80%	1.8	4.5	Saliva Cortisol (Afternoon)	20%	90%	0.4	1.5
Estriol (E3)	20%	80%	5	18	Saliva Cortisol (Night)	0	95%	0	0.9
2-OH-E1	20%	80%	5.1	13.1	Saliva Cortisol (2-3 am)	0	90%	0	0.9
4-OH-E1	0	80%	0	1.8	Saliva Cortisone Waking (W)	20%	90%	6.8	14.5
16-OH-E1	20%	80%	0.7	2.6	Saliva Cortisone (W+30 min.)	20%	90%	12.4	19.4
2-Methoxy-E1	20%	80%	2.5	6.5	Saliva Cortisone (W+60 min.)	20%	90%	9.4	15.3
2-OH-E2	0	80%	0	1.2	Saliva Cortisone Afternoon	20%	90%	2	7.1
4-OH-E2	20%	80%	0	0.5	Saliva Cortisone Night	0	95%	0	4.8
2-16-ratio	20%	80%	2.69	11.83	Saliva Cortisone (2-3 am)	0	95%	0	4.8
2-4-ratio	20%	80%	5.4	12.62	Melatonin (6-OHMS)	20%	90%	10	85
2Me-2OH-ratio	20%	80%	0.39	0.67	8-OHdG	0	90%	0	5.2
DHEA-S	20%	90%	20	750	Methylmalonate	0	90%	0	2.5
Androsterone	20%	80%	200	1650	Xanthurenate	0	90%	0.12	1.2
Etiocholanolone	20%	80%	200	1000	Kynurenate	0	90%	0.8	4.5
Testosterone	20%	80%	2.3	14	b-Hydroxyisovalerate	0	90%	0	12.5
5a-DHT	0	80%	0	6.6	Pyroglutamate	10%	90%	28	58
5a-Androstenediol	20%	80%	6	30	Indican	0	90%	0	100
5b-Androstenediol	20%	80%	20	75	Homovanillate	10%	95%	3	11
Epi-Testosterone	20%	80%	2.3	14	Vanilmandelate	10%	95%	2.2	5.5
a-THF	20%	90%	75	370	Quinolinate	0	90%	0	9.6
b-THF	20%	90%	1050	2500					
b-THE	20%	90%	1550	3800					
					Calculated Values				
					Total DHEA Production	20%	80%	500	3000
					Total Estrogens	20%	80%	35	70
					Metabolized Cortisol	20%	90%	2750	6500
					Saliva Cortisol Total	20%	90%	9.6	19.3
					Saliva Cortisone Total	20%	90%	36	55
<p>% = 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."</p>									