

TEST NUMBER: G-NL-XXXXX
GENDER: XXXXXX
AGE: XX

COLLECTED: 00-XXX-2024
RECEIVED: 00-XXX-2024

TESTED:

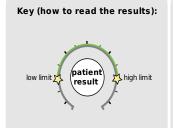
00-XXX-2024

TEST REF: GNL-NL-XXXXX

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TEST NAME: DUTCH Plus™

Hormone Testing Summary

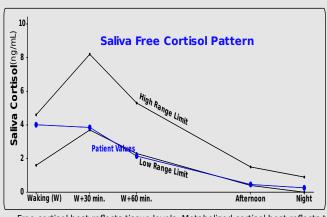






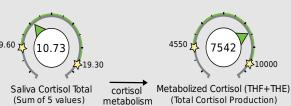
Testo	sterone
Age	Range
18-25	35-115
26-40	30-95
41-60	25-80
>60	20-60

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.

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

- The patient reports using a glucocorticoid. This may suppress adrenal function. See provider (
The Cortisol Awakening Response (CAR) actually showed a decrease in this case. Normal results show an increase of 50-160%. See page 5.

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Category	Test		Result	Units	Normal Range
Progesto	erone Metabolites (Urine)				
	b-Pregnanediol	Within range	119.8	ng/mg	75 - 400
	a-Pregnanediol	Below range	17.6	ng/mg	20 - 130
Estroge	ns and Metabolites (Urine)				
	Estrone(E1)	Above range	16.13	ng/mg	4 - 16
	Estradiol(E2)	Above range	2.26	ng/mg	0.5 - 2.2
	Estriol(E3)	Within range	3.7	ng/mg	2 - 8
	2-OH-E1	Within range	4.88	ng/mg	0 - 5.9
	4-OH-E1	Above range	1.44	ng/mg	0 - 0.8
	16-OH-E1	Within range	0.42	ng/mg	0 - 1.2
	2-Methoxy-E1	High end of range	2.76	ng/mg	0 - 2.8
	2-OH-E2	Within range	0.21	ng/mg	0 - 0.6
	4-OH-E2	High end of range	0.27	ng/mg	0 - 0.3
	Total Estrogen	High end of range	32.1	ng/mg	10 - 34
Metabol	ite Ratios				
	2-OH / 16-OH-E1 Balance	Above range	11.63	ratio	2.85 - 9.88
	2-OH / 4-OH-E1 Balance	Below range	3.39	ratio	6.44 - 12.6
	2-Methoxy / 2-OH Balance	Within range	0.57	ratio	0.4 - 0.7
Androge	ns and Metabolites (Urine)				
	DHEA-S	Low end of range	49.2	ng/mg	30 - 1500
	Androsterone	Within range	1712.5	ng/mg	500 - 3000
	Etiocholanolone	Low end of range	552.0	ng/mg	400 - 1500
	Testosterone	Within range	74.84	ng/mg	25 - 115
	5a-DHT	Within range	14.3	ng/mg	5 - 25
	5a-Androstanediol	Low end of range	65.3	ng/mg	30 - 250
	5b-Androstanediol	Low end of range	44.3	ng/mg	40 - 250
	Epi-Testosterone	Within range	56.0	ng/mg	25 - 115



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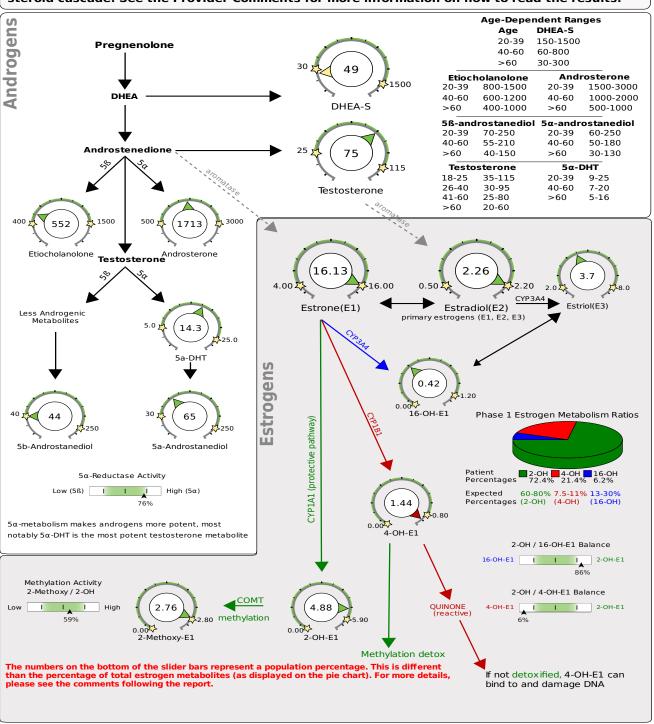
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Hormone metabolite results from the previous page are presented here as they are found in the steroid cascade. See the Provider Comments for more information on how to read the results.



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Category	Test		Result	Units	Normal Range
Free Cortis	sol and Cortisone (Saliva)				
	Saliva Cortisol - Waking (W)	Within range	4.01	ng/mL	1.6 - 4.6
	Saliva Cortisol - W+30 min.	Low end of range	3.85	ng/mL	3.7 - 8.2
	Saliva Cortisol - W+60 min.	Below range	2.15	ng/mL	2.3 - 5.3
	Saliva Cortisol - Afternoon	Low end of range	0.46	ng/mL	0.4 - 1.5
	Saliva Cortisol - Night	Within range	0.26	ng/mL	0 - 0.9
	Saliva Cortisone - Waking (W)	Within range	11.78	ng/mL	6.8 - 14.5
	Saliva Cortisone - W+30 min.	Within range	15.91	ng/mL	12.4 - 19.4
	Saliva Cortisone - W+60 min.	Within range	11.29	ng/mL	9.4 - 15.3
	Saliva Cortisone - Afternoon	Within range	4.22	ng/mL	2 - 7.1
	Saliva Cortisone - Night	Within range	2.71	ng/mL	0 - 4.8
	Saliva Cortisol Total	Low end of range	10.73	ng/mL	9.6 - 19.3
	Saliva Cortisone Total	Within range	45.91	ng/mL	36 - 55
Creatinine	(Urine)				
	Creatinine A (Waking)	Within range	0.49	mg/ml	0.3 - 3
	Creatinine B (Morning)	Within range	0.61	mg/ml	0.3 - 3
	Creatinine C (Afternoon)	Within range	0.63	mg/ml	0.3 - 3
	Creatinine D (Night)	Within range	0.84	mg/ml	0.3 - 3
Cortisol Mo	etabolites and DHEA-S (Urine)				
	a-Tetrahydrocortisol (a-THF)	Above range	1219.5	ng/mg	175 - 700
	b-Tetrahydrocortisol (b-THF)	Within range	3004.8	ng/mg	1750 - 4000
	b-Tetrahydrocortisone (b-THE)	Within range	3317.4	ng/mg	2350 - 5800
	Metabolized Cortisol (THF+THE)	Within range	7541.7	ng/mg	4550 - 10000
	DHEA-S	Low end of range	49.2	ng/mg	30 - 1500



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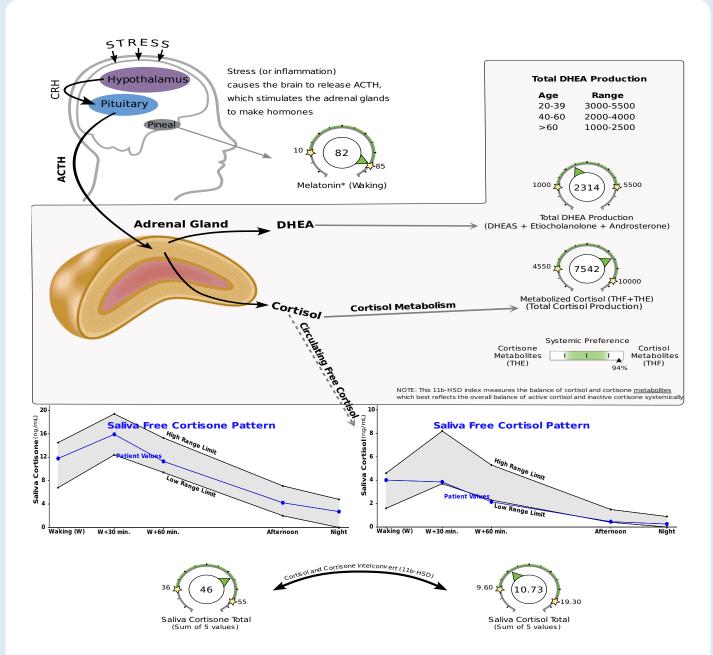
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PRACTITIONER:

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TEST NAME: DUTCH Plus™



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 4.01 and was actually lower at 3.85 after 30.0 minutes. This implies potential dysfunction in the HPA-axis or possibly improper collection. Expected increases differ depending on the methods used. Preliminary research shows that 50-160% or 1.5-4.0ng/mL increases are common. These guidelines are considered research only. This patient shows a salivary cortisol of 2.15 measured 60 minutes after waking. Generally this result is a little higher than the waking sample but is not in this case. To date, data suggests that expected results may be 0-70% higher, and this guideline is considered for research only.



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Category	Test		Result	Units	Normal Range					
	Nu	tritional Organic Acid	ls							
Vitamin B12 N	Marker (may be deficient if high) - (Urine)								
	Methylmalonate (MMA)	Within range	1.4	ug/mg	0 - 3.5					
Vitamin B6 M	Vitamin B6 Markers (may be deficient if high) - (Urine)									
	Xanthurenate	Within range	0.49	ug/mg	0.2 - 1.9					
	Kynurenate	Within range	3.4	ug/mg	1 - 6.6					
Biotin Marker	(may be deficient if high) - (Urin	ne)								
	b-Hydroxyisovalerate	Within range	8.2	ug/mg	0 - 18					
Glutathione M	larker (may be deficient if low o	r high) - (Urine)								
	Pyroglutamate	Within range	53.9	ug/mg	38 - 83					
Gut Marker (potential gut putrefaction or dysbiosis if high) - (Urine)										
	Indican	Within range	46.5	ug/mg	0 - 131					
	N	euro-related Markers	}							
Dopamine Me	etabolite - (Urine)									
	Homovanillate (HVA)	Within range	6.1	ug/mg	4 - 16					
Norepinephrir	ne/Epinephrine Metabolite - (Uri	ne)								
	Vanilmandelate (VMA)	Within range	5.2	ug/mg	2.5 - 7.5					
Neuroinflamn	nation Marker - (Urine)									
	Quinolinate	Within range	8.6	ug/mg	0 - 12.5					
		Additional Markers								
Melatonin (*n	neasured as 6-OH-Melatonin-Sເ	ılfate) - (Urine)								
	Melatonin* (Waking)	High end of range	81.8	ng/mg	10 - 85					
Oxidative Stre	ess / DNA Damage, measured a		anosine (8	-OHdG) -	(Urine)					
	8-OHdG (Waking)	Within range	1.3	ng/mg	0 - 8.8					



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Clinical Support Overview

Please note: This is an amended report. Please discard any previous download of this report. Ifyou have questions about the changes, please call the lab.

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How to read the DUTCH report

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

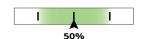
DUTCH Dials

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 reported symptoms of excess estrogen. This can be caused by excess estrogen or progesterone deficiency. Results should be carefully reviewed. We do not report a progesterone to estrogen ratio. However, you can investigate this issue by looking at the relative level of these two hormones on their respective dials.

The patient reports significant symptoms of androgen deficiency.

Androgen Metabolism

Androgen Metabolites: DHEA

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

DHEA peaks for men in their 20'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 aromatize to estrone. DHEA-s is made by sulfation, has a much longer half-life than DHEA and 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.

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The Total DHEA Production (page 1) was about 2,314ng/mg which is within the overall range and also within the age-dependent range for this patient. This implies that the adrenal glands are producing appropriate DHEA levels.

• 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.

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.

The testes make most of the male's testosterone. Levels tend to be their highest at around 20 years of age and start to decline when men get into their 30's. Levels continue to drop as men age. Testosterone is needed for building bones and muscle mass, regulating body fat distribution and in the production of sperm and red blood cells. Testosterone is also important for libido and downstream production of modest amounts of estrogen.

Age dependent ranges are provided for all androgens as some decline is seen with age. Testosterone levels in healthy men vary widely so it is suggested that these ranges be interpreted with caution and consideration of symptoms. In addition, because estrogen also supports libido, erections and healthy weight management, estrogen levels should be considered along with the testosterone levels when assessing symptoms.

The testosterone result 74.8ng/mg is above the age dependent range. Review the levels of all androgens, androgenic metabolism, patient's therapies, and symptoms for a complete assessment.

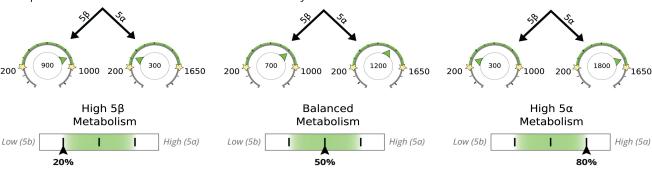
• Andogen Metabolites: 5a-reductase versus 5b-reductase

5a-reductase converts testosterone into 5a-DHT (DHT), which is even more potent (\sim 3x) than testosterone. High levels of DHT can lead to symptoms associated with too much testosterone (thinning scalp hair, acne, etc.) and may also be associated with prostate issues in older men. However, 5aDHT plays an integral role in supporting bone, muscle and connective tissue integrity and improving brain health through the upregulation of dopamine, which can improve mood and libido.

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 the balance between etiocholanolone (5b) and androsterone (5a) as well as 5a-androstanediol and 5b-androstanediol. 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 total androgen levels if high androgen symptoms are a concern.

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



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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 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.

Estrogen Metabolism

Over the past few decades research has clarified the importance of healthy estrogen levels and a balanced estrogen to testosterone ratio in men. The testes produce approximately 20% of E2 (Rochira) and the remaining 80% is aromatized from androgens in adipose (fat) tissue, muscle, breast, brain, liver and bone (Rochira). Thus, most of the estrogen in men is aromatized from testosterone, androstenedione, and DHEA in the periphery. The three estrogens (in order of strongest to weakest) are: Estradiol (E2), Estrone (E1) and Estriol (E3). E1 and E2 can interconvert and E3 is a waste product of estradiol and is the weakest of the three estrogens.

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

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

Levels of the primary estrogen, estradiol (the strongest estrogen), as well as "total estrogens" may be considered.

• 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.

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.

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.

Estrogens were above range. High estrogen in men may be due to elevated testosterone, obesity, blood sugar and insulin dysregulation, inflammation, poor detoxification, EDCs (endocrine disrupting chemicals), alcohol and oral/sublingual estrogen, DHEA and testosterone supplementation. Use caution when interpreting estrogen results if estrogen, DHEA or testosterone was supplemented orally or sublingually, as these undergo first pass metabolism which results in elevated urinary metabolites that do not correlate with serum levels.

Elevated estrogen has been associated with enlargement of breast tissue, weight gain, mild hypogonadotropic hypogonadism, mood swings, low sex drive and impaired erectile function. Besides assessing and treating the cause, other treatments include blocking aromatase with medications such as anastrozole or naturally with supplements such as topical chrysin, oral damiana, mangosteen and Agaricus (white button mushroom).

Progesterone Metabolism

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Male progesterone is synthesized in the testes and, to a lesser degree, in the adrenal glands. It's role in men's health is not well understood, although progesterone is known to be involved in sperm activation. In healthy men, progesterone is positively correlated to markers of inflammation.

Metabolites of progesterone are measured in urine, including 5b-pregnanediol and 5a-pregnanediol. 5bpregnanediol is inactive in the body but is the major metabolite of progesterone. 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. Both taken together represent the major metabolic end points for progesterone and can be used to represent total progesterone production.

The weighted average of the two progesterone metabolites shows progesterone is low. In males, the significance of progesterone is not well understood. Low progesterone might be an artifact of low overall testicular or adrenal hormone production. Treatment might include support for testosterone such as maca, ashwagandha, Tribulus, fenugreek, zinc and vitamin C.

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, 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.

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• 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 Áwakening Response is measured as a percent difference between the waking and 30-minute (peak) 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.

The patient reported using a glucocorticoid medication. Please be aware that glucocorticoids can suppress the ACTH signal, resulting in a suppressed adrenal pattern. This effect is heavily influenced by the type of glucocorticoid, the dose and the duration of use. Other adrenal hormones such as DHEA, androgens and mineralocorticoids may also be suppressed. It is not known if natural supplements, such as adaptogens, can overcome adrenal suppression from continued glucocorticoid use. Please keep this in mind when evaluating these results.

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

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TEST NAME: DUTCH Plus™

highest concentration in muscle tissue.

not established in the scientific literature.

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.

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

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

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

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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.

Quinolinate (QA)

The quinolinate is high. Quinolinate is a neurotoxin derived from tryptophan metabolism in the context of high inflammation or high cortisol. Elevated quinolinate 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 quinolinate 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 quinolinate 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.

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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.

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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:

	300	700	Male	Referenc	e Ranges (Updated 05.1.2024)	100		10	
	Low%	High%	Low	High		Low%	High%	Low	High
b-Pregnanediol	10%	90%	75	400	Saliva Cortisol Waking (W)	20%	90%	1.6	4.6
a-Pregnanediol	10%	90%	20	130	Saliva Cortisol (W+30 min.)	20%	90%	3.7	8.2
Estrone (E1)	10%	90%	4	16	Saliva Cortisol (W+60 min.)	20%	90%	2.3	5.3
Estradiol (E2)	10%	90%	0.5	2.2	Saliva Cortisol (Afternoon)	20%	90%	0.4	1.5
Estriol (E3)	10%	90%	2	8	Saliva Cortisol (Night)	0	95%	0	0.9
2-OH-E1	0	90%	0	5.9	Saliva Cortisol (2-3 am)	0	90%	0	0.9
4-OH-E1	0	90%	0	0.8	Saliva Cortisone Waking (W)	20%	90%	6.8	14.5
16-OH-E1	0	90%	0	1.2	Saliva Cortisone (W+30 min.)	20%	90%	12.4	19.4
2-Methoxy-E1	0	90%	0	2.8	Saliva Cortisone (W+60 min.)	20%	90%	9.4	15.3
2-OH-E2	0	90%	0	0.6	Saliva Cortisone Afternoon	20%	90%	2	7.1
4-OH-E2	0	90%	0	0.3	Saliva Cortisone Night	0	95%	0	4.8
2-16-ratio	20%	80%	2.85	9.88	Saliva Cortisone (2-3 am)	0	95%	0	4.8
2-4-ratio	20%	80%	6.44	12.6	Melatonin (6-OHMS)	20%	90%	10	85
2Me-2OH-ratio	20%	80%	0.4	0.7	8-OHdG	0	90%	0	8.8
DHEA-S	20%	90%	30	1500	Methylmalonate	0	90%	0	3.5
Androsterone	20%	80%	500	3000	Xanthurenate	0	90%	0.2	1.9
Etiocholanolone	20%	80%	400	1500	Kynurenate	0	90%	1	6.6
Testosterone	20%	90%	25	115	b-Hydroxyisovalerate	0	90%	0	18
5a-DHT	20%	90%	5	25	Pyroglutamate	10%	90%	38	83
5a-Androstanediol	20%	90%	30	250	Indican	0	90%	0	131
5b-Androstanediol	20%	90%	40	250	Homovanillate	10%	95%	4	16
Epi-Testosterone	20%	90%	25	115	Vanilmandelate	10%	95%	2.5	7.5
a-THF	20%	90%	175	700	Quinolinate	0	90%	0	12.5
b-THF	20%	90%	1750	4000					
b-THE	20%	90%	2350	5800	Calculated Values	j			1
					Total DHEA Production	20%	80%	1000	5500
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results higher than s					Metabolized Cortisol	20%	90%	4550	10000
	will be desi			ererence	Saliva Cortisol Total	20%	90%	9.6	19.3
range	witt be desi	griated as T	ngn.		Saliva Cortisone Total	20%	90%	36	55

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TEST NAME: DUTCH Plus™

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TEST NAME: DUTCH Plus™

		Win-	I do not suspect I have this	I suspect I may have this	/ I have diagnosed		Nordic Laboratories
S	Addison's Dise	ease					200010100
ISEASE STATES	Adrenal Fatigu	ie					
⋖	Chronic Fatigue						Nygade 6. 3 Sal, Copenhagen, Denmark
2	Cushing's Disease						+45 33 75 1000 info@nordic-labs.com
B I	High Blood Pr	th Blood Pressure			Please List any Current/Recent		
A I	Hyperthyroidi	sm (Overa	active)				Medical Diagnosis Not Listed
S	Hypothyroidis						Elsewhere On This Form
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GUE	0 = Never/No	THE REAL PROPERTY.		0 1	2 3		
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≥ ≥	0 = Never/No	20	Trouble Falling	Asleep \Box			☐ Underweight
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- F	lelp miet richtry to	Lof	fatigue se my or	reluxi verwe	ing .	THIS TEST WILL HEL	
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