



DUTCH Caveats

WHAT YOU NEED TO KNOW WHEN USING DUTCH
by
Mark Newman, MS – President/Founder of Precision Analytical



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Clinical Biochemistry
Volume 46, Issues 13-14, September 2013, Pages 1175-1179



The cost of poor blood specimen quality and errors in preanalytical processes

LAB TESTING ALWAYS CARRIES WITH IT SOME **UNCERTAINTY**

- Even when the lab gets it exactly right...
- “Mistakes made prior to samples being tested comprise >70% of lab-related errors.”



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DUTCH Strengths

- Comprehensive




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Dried Urine **Se** **DUTCH CAVEAT #1** **Saliva**

This does NOT include xenoestrogens

- Estrogen (production)
- Estrogen (metabolism)
- Estrogen (methylation)
- Testosterone (production)
- Testosterone (metabolism)
- DHEA (production)
- DHEA (metabolites)
- Progesterone (production)
- Cortisol (free pattern)
- Cortisol (production)
- Cortisol (metabolism)
- B6, B12, Glut. deficiency
- Neurotransmitter balance
- Oxidative stress
- Melatonin (production)


- Testosterone (metabolism)
- DHEA (production)
- Progesterone (production)
- Cortisol (free pattern)
- Cortisol (CAR)



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DUTCH Strengths

- Comprehensive
- Easy collection
- Represents approximate 24-hour period
- Possibly the best overall HRT monitoring tool



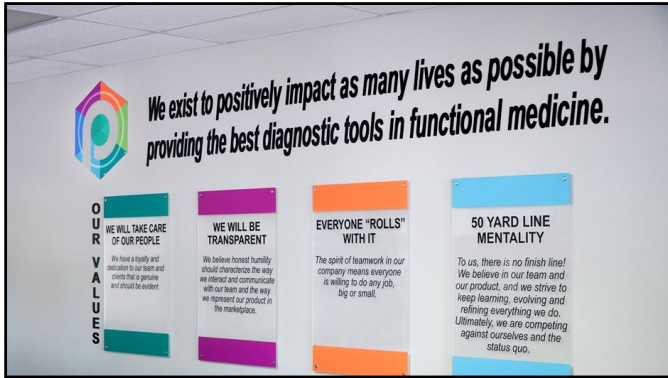
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DUTCH Caveats

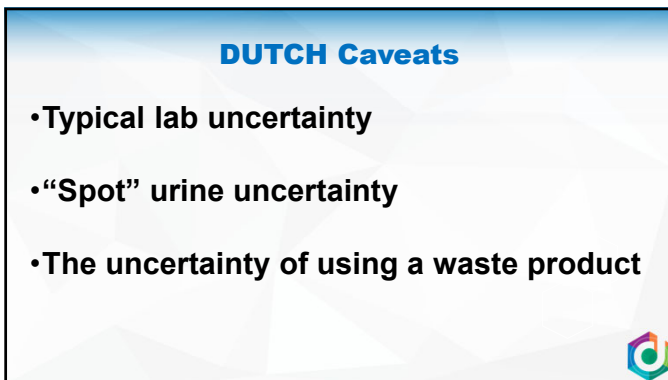
Where is the greatest **uncertainty** with DUTCH?



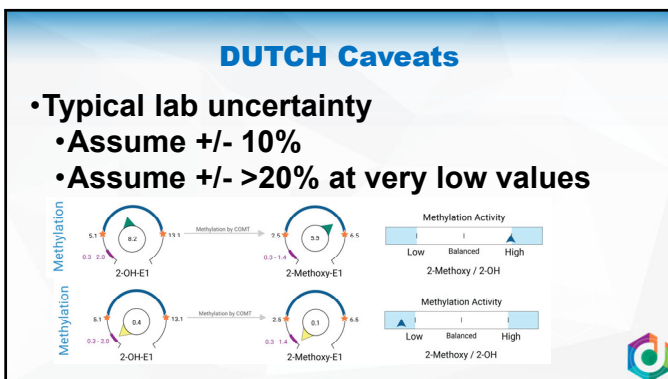
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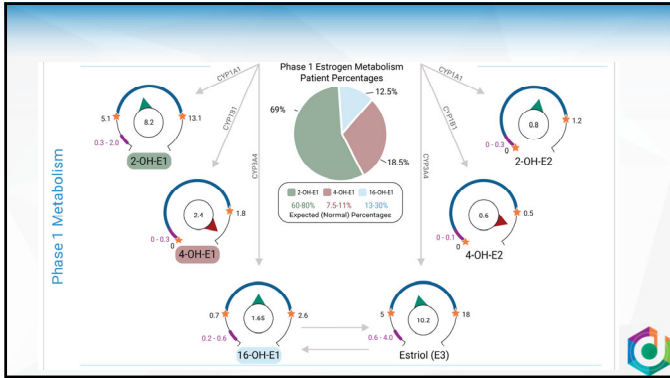
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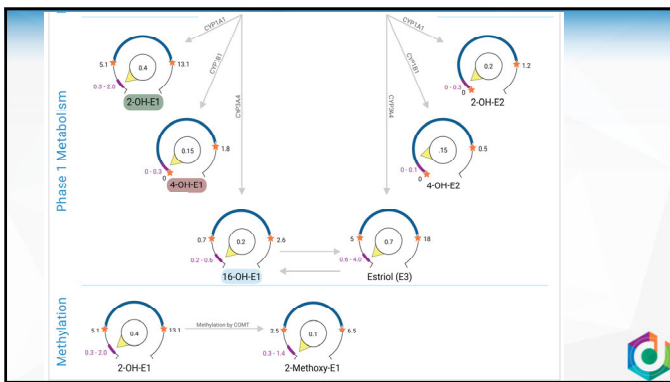
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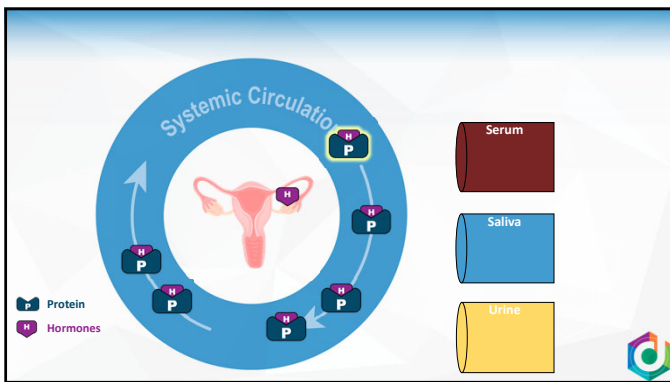
DUTCH Caveats

- Typical lab uncertainty
- **“Spot” urine uncertainty**
- The uncertainty of using a waste product

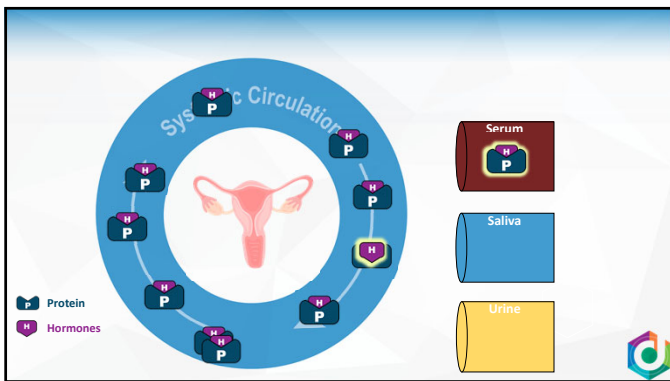
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How Does Urine Testing Work?

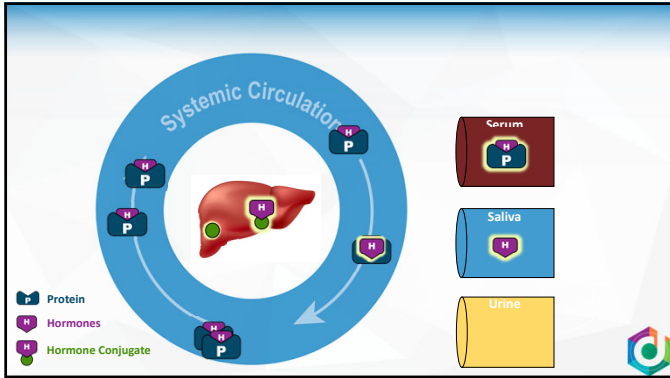
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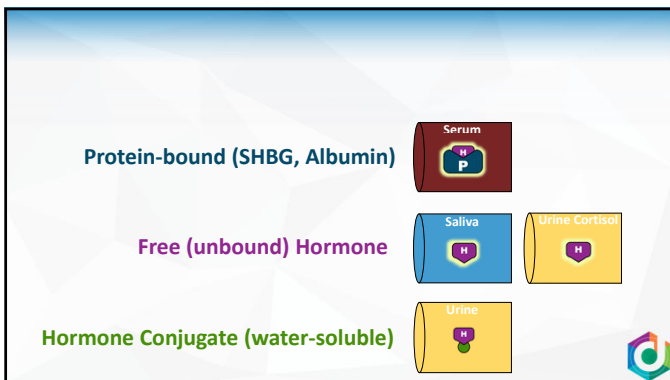
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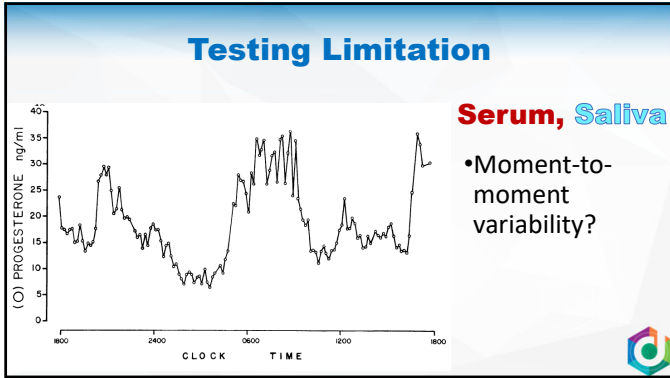


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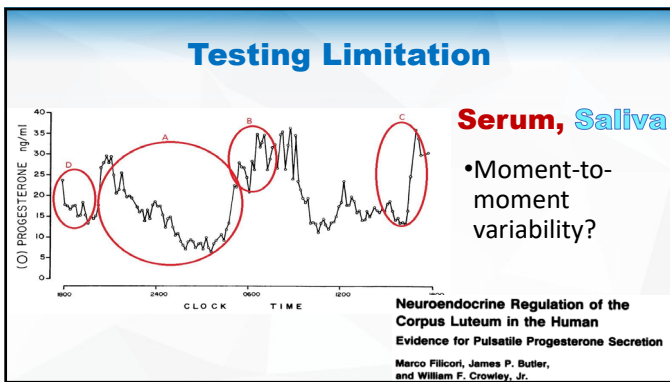
DUTCH Caveats

- **“Spot” urine weaknesses**
 - Creatinine dependence
- **Urine general weaknesses**
 - It's a waste product!

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Testing Limitation


<p>DUTCH</p> <ul style="list-style-type: none"> •Creatinine correction? 	<p>24Hr Urine</p> <ul style="list-style-type: none"> •Complete collection? •Sample measurement? 	<p>Serum, Saliva</p> <ul style="list-style-type: none"> •Moment-to-moment variability?
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Each test has its own drawbacks

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Creatinine Correction


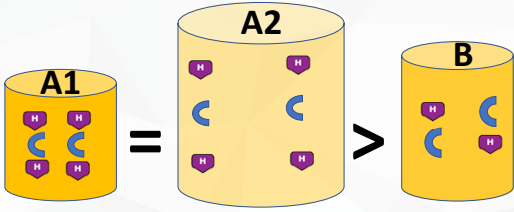
- Creatinine excretion is used to correct for hydration



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Creatinine Correction


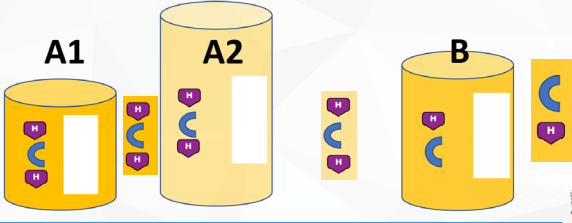
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Creatinine Correction

- Creatinine excretion is used to correct for hydration



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Creatinine Correction

•Creatinine excretion is used to correct for hydration

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dutchfest
2019

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Creatinine Correction

24-Hour Urine

University of Applied Sciences
dutchfest
2019

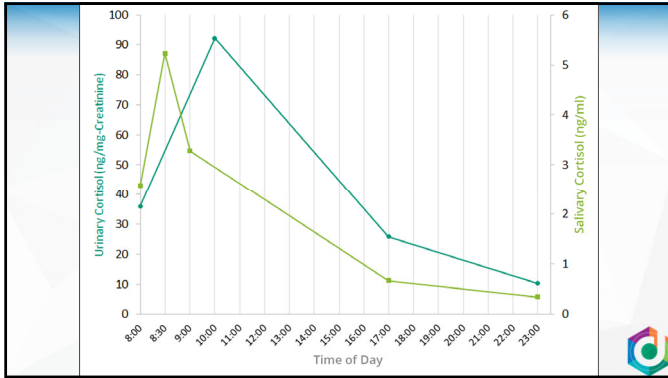
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Creatinine Correction

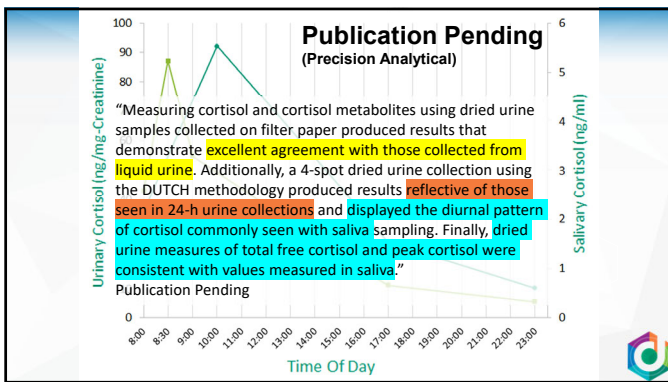
24-Hour Urine

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dutchfest
2019

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URINE CORTISOL CAVEAT

Never use urine for cortisol if the patient has a kidney issue

USE DUTCH PLUS!

Atypical CUR = Atypical urine cortisol excretion

If creatinine is very low (or high), individual results may be less certain

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URINE CORTISOL CAVEAT

Category	Test	Result	Units	Normal Range
Creatinine (Urine)	Creatinine A (Waking)	Within range	1.07 mg/ml	0.2 - 2
	Creatinine B (Morning)	Below range	0.1 mg/ml	0.2 - 2
	Creatinine C (Afternoon)	Within range	0.92 mg/ml	0.2 - 2
	Creatinine D (Night)	Above range	2.22 mg/ml	0.2 - 2

If creatinine is very low (or high), individual results may be less certain

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Daily Free Cortisone Pattern

Daily Free Cortisol Pattern

At least one sample has a very low creatinine (female <0.15; male <0.2, see page 41, and for these individual samples cortisol & cortisone values may be somewhat less reliable).

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Creatinine Correction

So, what's the problem?

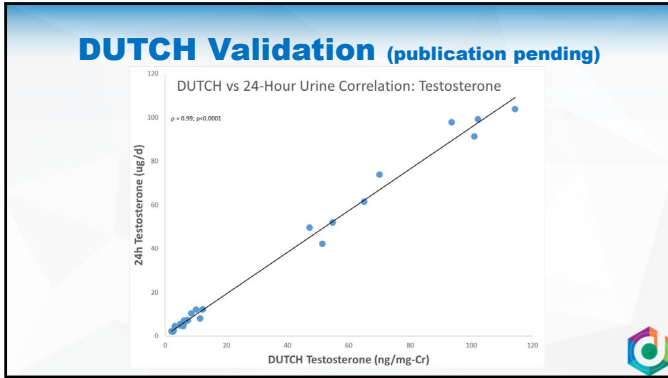
A (130lbs)

24Hr Urine
A=B

~~**Spot Urine**
A>B~~

B (240lbs)

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Creatinine Correction

- Creatinine excretion is used to correct for hydration
- Creatinine excretion is dependent on:
 - Age, Height, Weight
- Brings significant bias into "spot" urine testing
 - BUT, there is a "fix" to this problem

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Creatinine Correction


- Creatinine excretion is used to correct for hydration
- Bias due to person-to-person variability of creatinine

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Creatinine Correction

- Creatinine excretion is used to correct for hydration
- Bias due to person to person variability

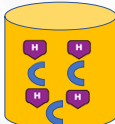

DUTCH
Fixed



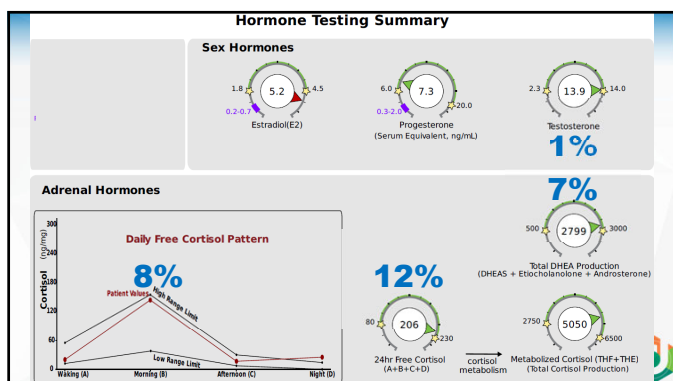
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Creatinine Correction

- Creatinine excretion is used to correct for hydration
- Bias IF the person doesn't excrete the predicted amount of 24hr creatinine
 - If this is an issue, it would impact any urine test reported "per creatinine" and would impact the entire profile equally.

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DUTCH Validation Data

- Basic Validation (precision, accuracy, etc.)
- 24 Hour Correlation
- Serum Correlation (E2, Pg)
- Saliva Correlation (cortisol, cortisone)

DUTCH PUBLISHED!
DUTCH PUBLISHED!
DUTCH PUBLISHED!
 (For E2/Pg)


PUBLICATION...PENDING!!!



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DUTCH Caveats

- Typical lab uncertainty
- “Spot” urine uncertainty
- The uncertainty of using a waste product



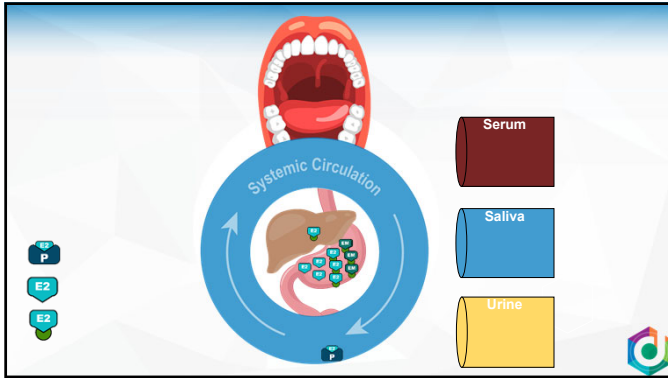
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DUTCH Caveats

- What about HRT?



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Urine Values are NOT Helpful for Dosing

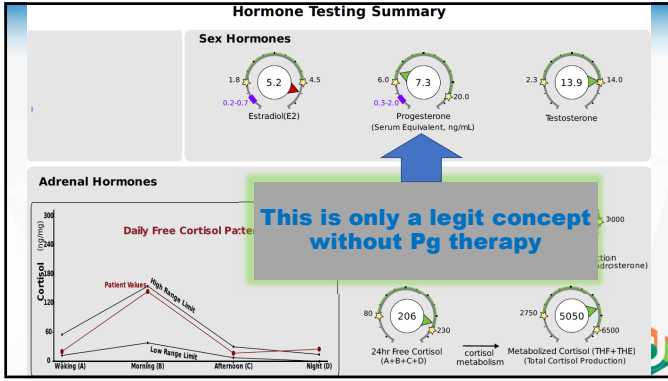
High results do NOT mean too much hormone

“Normal” results don’t mean you got it right

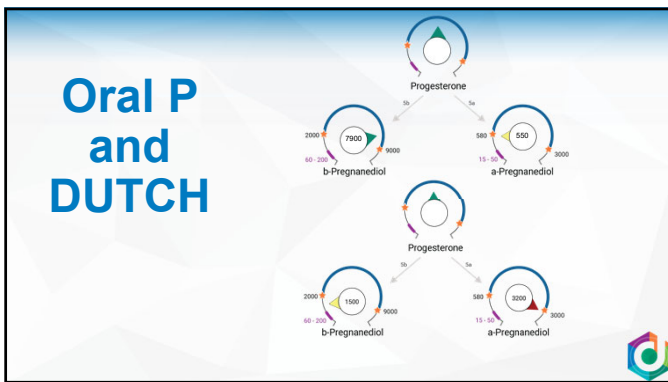
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Oral Progesterone	Patch, Pellet, Injection	Transdermal Estrogen	Transdermal Testosterone	Transdermal Progesterone	Vaginal or Anal Mucosa	Oral Estrogen	Sublingual
<p>✓ Dried Urine</p> <p>Dried urine provides useful feedback when using oral progesterone to aid sleep disturbance related to menopause. SA (more active) and SH (less active) metabolites are measured to individualize doses of oral progesterone. Much of the clinical impact is from the effects of the SAMeabolites.</p>	<p>✓ Dried Urine</p> <p>Values increase incrementally with dosing. For estrogen patches, oral progesterone, and oral progesterone, Pellets and injectors also increase levels incrementally, but the increase may exceed what is seen in serum testing. Dried urine allows for monitoring both the proper dosing of hormones as well as metabolic patterns.</p>	<p>✓ Dried Urine</p> <p>Target values between the top of the premenopausal range and the lower third of the perimenopausal range correlate with patient clinical improvement (bone density, hot flash relief, etc.). Doses that push levels to the middle of the premenopausal range and beyond may be excessive. Dried urine is preferred over serum because in addition to metabolites, dried urine averages out the up-and-down patterns found when using E2 gels and creams.</p>	<p>✓ Dried Urine</p> <p>Levels generally parallel measurable clinical outcomes (increased lean body mass, decreased LH values in men). Epi-testosterone values can also be used to assess gonadal suppression due to TRT (levels decrease as TRT increases and are ~10:1mg with complete suppression).</p>	<p>✗ Dried Urine</p> <p>Creams and gels cannot be effectively monitored with any lab testing. Values increase only slightly with dosing. Because of the uncertainty of tissue levels, take caution to use concurrently with estrogen therapy without endometrium surveillance (ultrasound or biopsy).</p>	<p>✓ Dried Urine (E1)</p> <p>Special method removes potential contamination and monitoring is helpful with most hormones. Very low doses may impact local tissue without increasing lab values.</p>	<p>✗ Dried Urine</p> <p>Cannot be used to effectively monitor dosing due to 1st-pass metabolism. Most of the hormone in urine has not been in circulation as "free" hormone.</p>	<p>✗ Dried Urine</p> <p>Lab testing is not effectively monitored with dried urine; metabolite patterns can be effectively assessed.</p>
<p>✗ SERUM</p> <p>Results go up-and-down quickly. If taken at bedtime, levels return to baseline within a few hours. Results can also be inaccurate due to progesterone metabolites cross-reacting with immunoassay tests.</p>	<p>✓ SERUM</p> <p>Serum testing is well suited for use with these types of therapies. Results increase with increased dosing in a fairly linear fashion.</p>	<p>✓ SERUM (gels)</p> <p>Effective for monitoring gels similar to patches but be aware of the up-and-down patterns, limiting interpretation.</p> <p>2 SERUM (creams)</p> <p>For E2 creams, limited published data shows a rapid up-and-down pattern questioning serum testing's reliability.</p>	<p>✓ SERUM</p> <p>Results correlate to clinical symptoms. In men, lean body mass increases only when serum and likely urine results increase.</p>	<p>✗ SERUM</p> <p>Values do not increase significantly with dosing.</p>	<p>✓ SERUM</p> <p>Progesterone is measured indirectly in urine by measuring pregnanediol. This metabolite is underrepresented when taken vaginally.</p>	<p>✓ SERUM</p> <p>While dosing is not effectively monitored with dried urine, metabolite patterns can be effectively assessed.</p>	<p>✗ SERUM</p> <p>While dosing is not effectively monitored with dried urine, metabolite patterns can be effectively assessed.</p>
				<p>✓ SERUM</p> <p>While serum levels likely represent systemic uptake of hormone, interpret with care as you may not know if your value represents a peak or a trough.</p>		<p>✓ SERUM</p> <p>Serum testing offers the best feedback on monitoring the actual dose of oral estradiol.</p>	<p>✗ SERUM</p> <p>Serum testing is not effective. Results come and fall too rapidly for useful testing. In many cases, results are back to baseline within a few hours.</p>

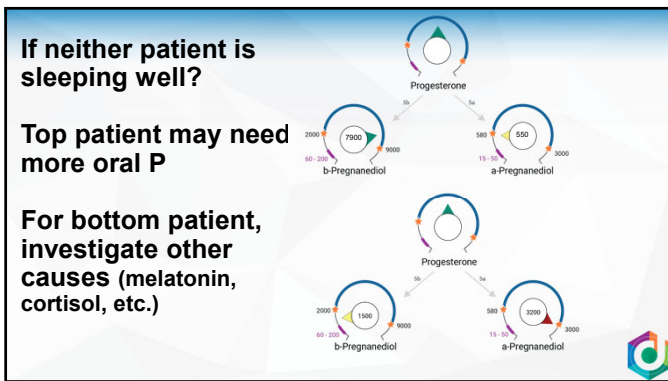
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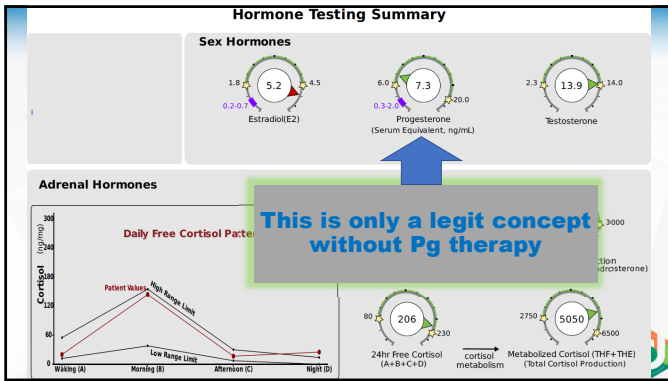
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DUTCH Caveats

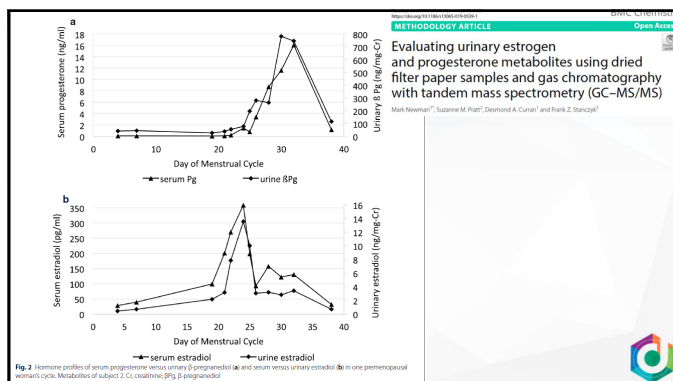
- Creatinine Dependence
- HRT?
- Phase 2 Metabolism Assumed
 - Testosterone has known issues that limit utility
 - More comprehensive panels make proper interpretation more likely

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Urine testing presupposes “normal” phase 2 metabolism. With most hormones, this assumption is almost always true

Hormone Conjugate (water-soluble)

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Newman et al. BMC Chemistry (2019) 13:20
<https://doi.org/10.1186/s13065-019-0539-1>

METHODOLOGY ARTICLE Open Access

Evaluating urinary estrogen and progesterone metabolites using dried filter paper samples and gas chromatography with tandem mass spectrometry (GC-MS/MS)

Mark Newman^{1*}, Suzanne M. Pratt², Desmond A. Curran¹ and Frank Z. Stanczyk³

Abstract
Background: Measuring concentrations of metabolites of estradiol and progesterone in urine, instead of measuring serum concentrations, is common in research and also used in patient care. The primary aim of this study was to
Conclusions: For estradiol and progesterone, the dried urine assay is a good surrogate for serum testing. The 4-spot method can be used instead of 24-h urine collections and dried urine results are comparable to liquid urine. The dried urine assay is useful for some clinical assessments of hormone disorders and may be useful in large epidemiologic studies due to ease of sample handling.

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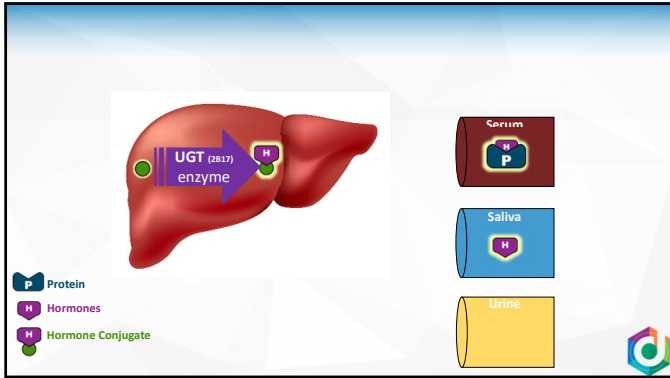
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Urine testing presupposes “normal” phase 2 metabolism. With most hormones, this assumption is almost always true

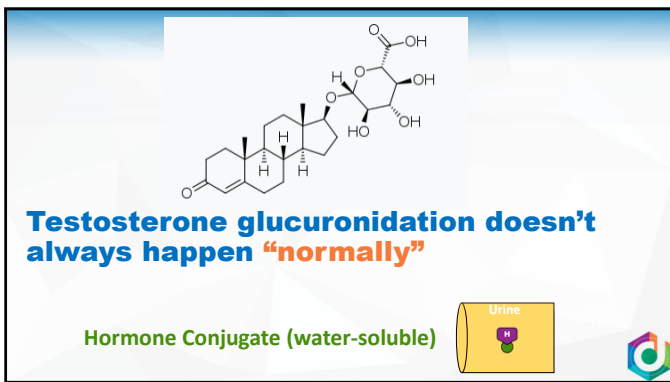
Testosterone glucuronidation doesn't always happen “normally”

Hormone Conjugate (water-soluble)

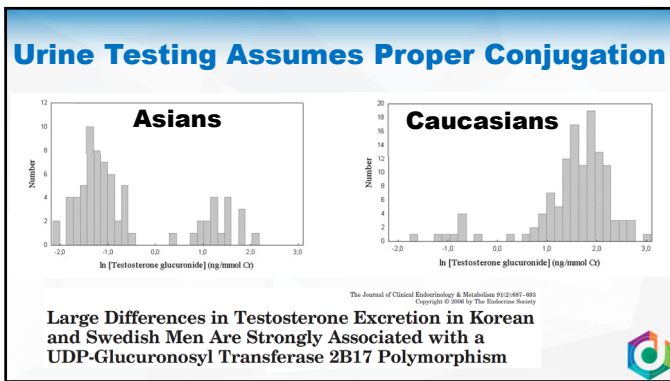
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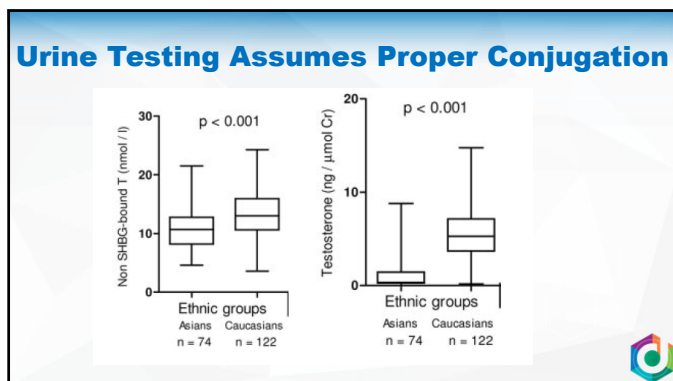
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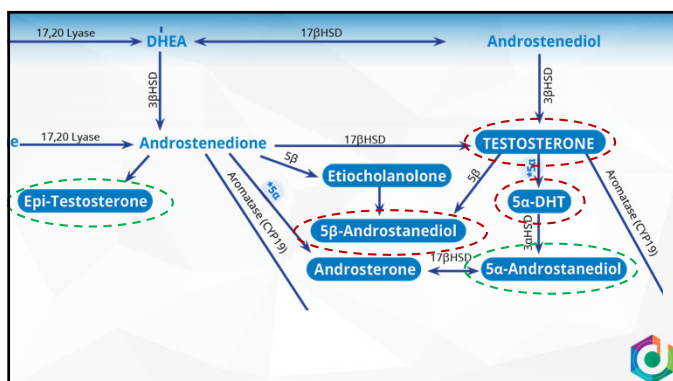
UGT Defect (Variant) – Who has it?

- >60% of people of Asian descent
- <10% of other ethnicities
- Caused by a gene deletion
- No known physiological consequence

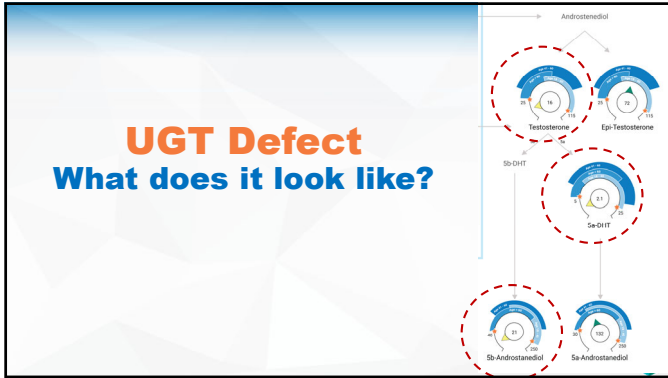
The Journal of Clinical Endocrinology & Metabolism 91(2):607–609
Copyright © 2006 by The Endocrine Society

Large Differences in Testosterone Excretion in Korean and Swedish Men Are Strongly Associated with a UDP-Glucuronosyl Transferase 2B17 Polymorphism

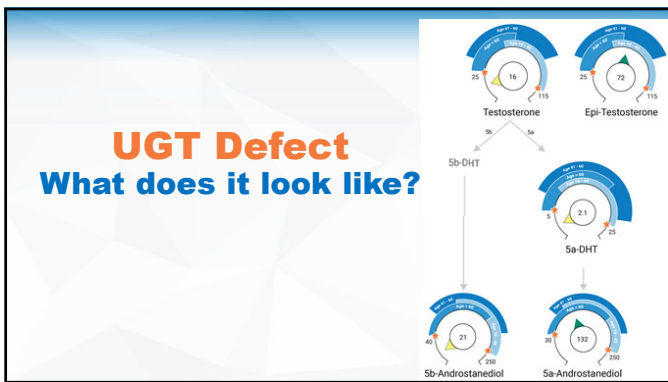
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UGT Defect
What does it look like?

- **Testosterone** (only in urine) is falsely low
- **EpiT** and **5a-androstanediol** are “right”
- **DHT** and **5b-androstanediol** are also falsely low

• Why? T, DHT, 5b-Androstanediol are conjugated by the same enzyme (different for epiT)

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Urine Pros and Cons

- + A better average over time than serum/saliva
- +GC-MS or LC-MS can be more accurate at low levels compared to some serum/saliva (EIA)
- + Reflects bioavailable hormone, but only IF phase 2 metabolism is appropriate

- Assumes phase 2 metabolism is appropriate



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So WHAT? For Patients not on TRT

- Urine is a useful secondary test for T
 - Serum is primary
- Urine is not reliable for patients of Asian descent
- When Epi-T > T, be suspicious
 - Especially if DHT, b-androstenediol are also low
- Confirm low T with serum before TRT



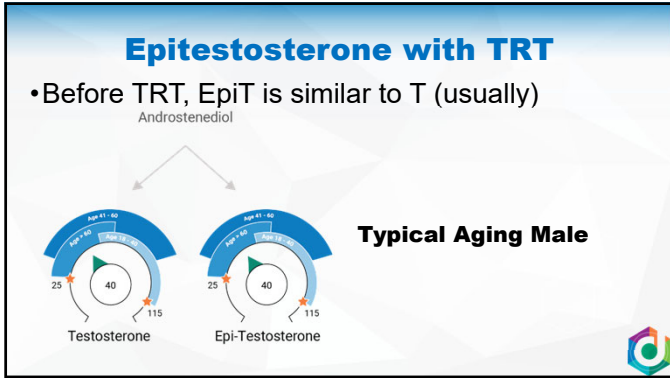
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So WHAT? For Patients on TRT

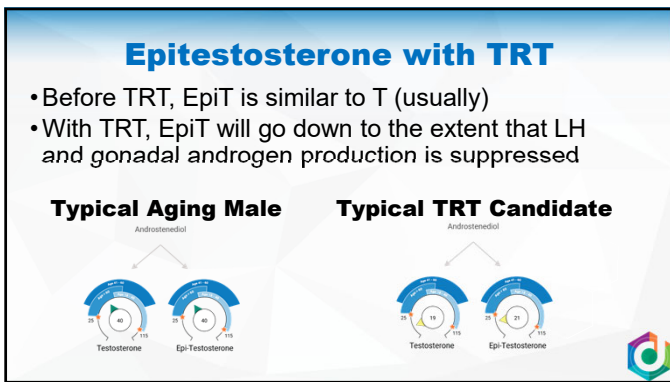
- Urine is a useful secondary test for T
 - Serum is primary
- Urine is not reliable for patients of Asian descent
- **Use Epi-T as a surrogate for approximate testicular production of T in men**



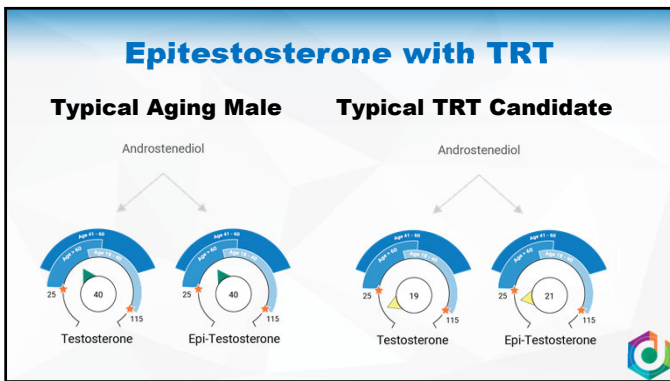
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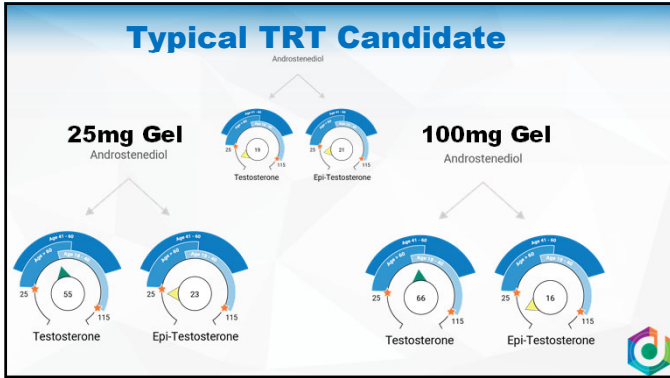
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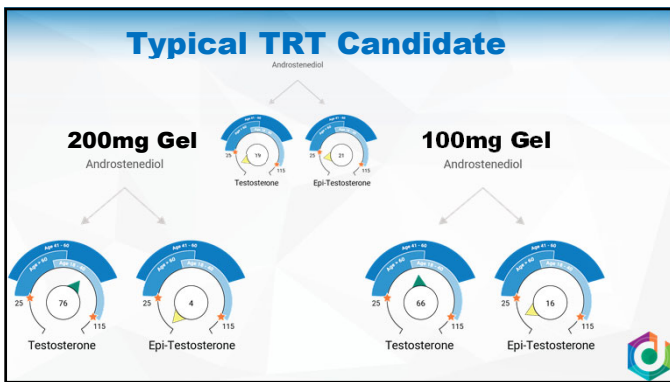
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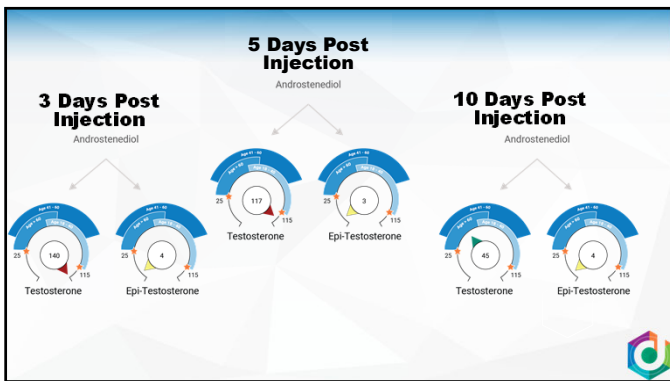
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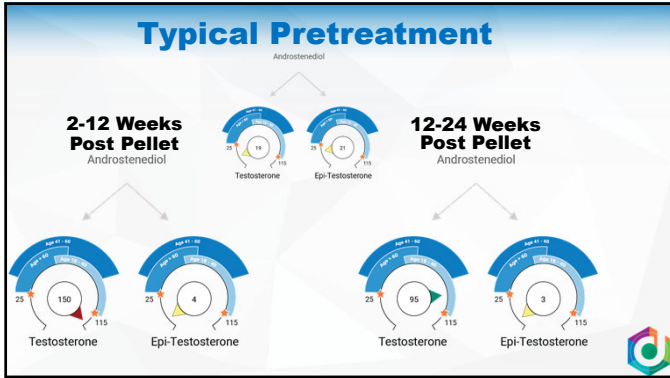
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Epitestosterone, A Useful Tool

Testosterone

Epitestosterone

- Helps identify the UGT variant
- Approximates gonadal androgen production for:
 - UGT+ (falsely low urine T)
 - TRT Patients

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DUTCH Caveats

- **Creatinine Dependence**
 - Creatinine dependence
- **A few select HRT**
- **Phase 2 Metabolism Assumed**
 - Testosterone has known issues that limit utility
 - More comprehensive panels make proper interpretation more likely

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DUTCH Caveats

- When to not use
 - Kidney issue
 - Asian descent if focusing on testo or TRT
 - If wanting to monitor dosing for oral E2, sublingual hormones, etc.



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DUTCH Caveats


- **When to not use**
 - Kidney issue
 - Asian descent if focusing on testo or TRT
 - If wanting to monitor dosing for oral E2, sublingual hormones, etc.
 - If on TRT and only interested in T levels



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DUTCH Caveats


- **When to be careful with interpretation**
 - When urine T is low and Epi-T is higher
 - Very low or very high creatinine
 - Especially individual cortisol results
 - Ratio analysis (2/16) at very low concentrations



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DUTCH Caveats


- **When to be careful with interpretation**
 - Results that may be artificially low or elevated
 - How do I know?
 - IF THE CLINICAL PICTURE AND LABS DON'T MATCH! – this should be true of any lab



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Thank you!

Lecture questions?
info@dutchtest.com



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