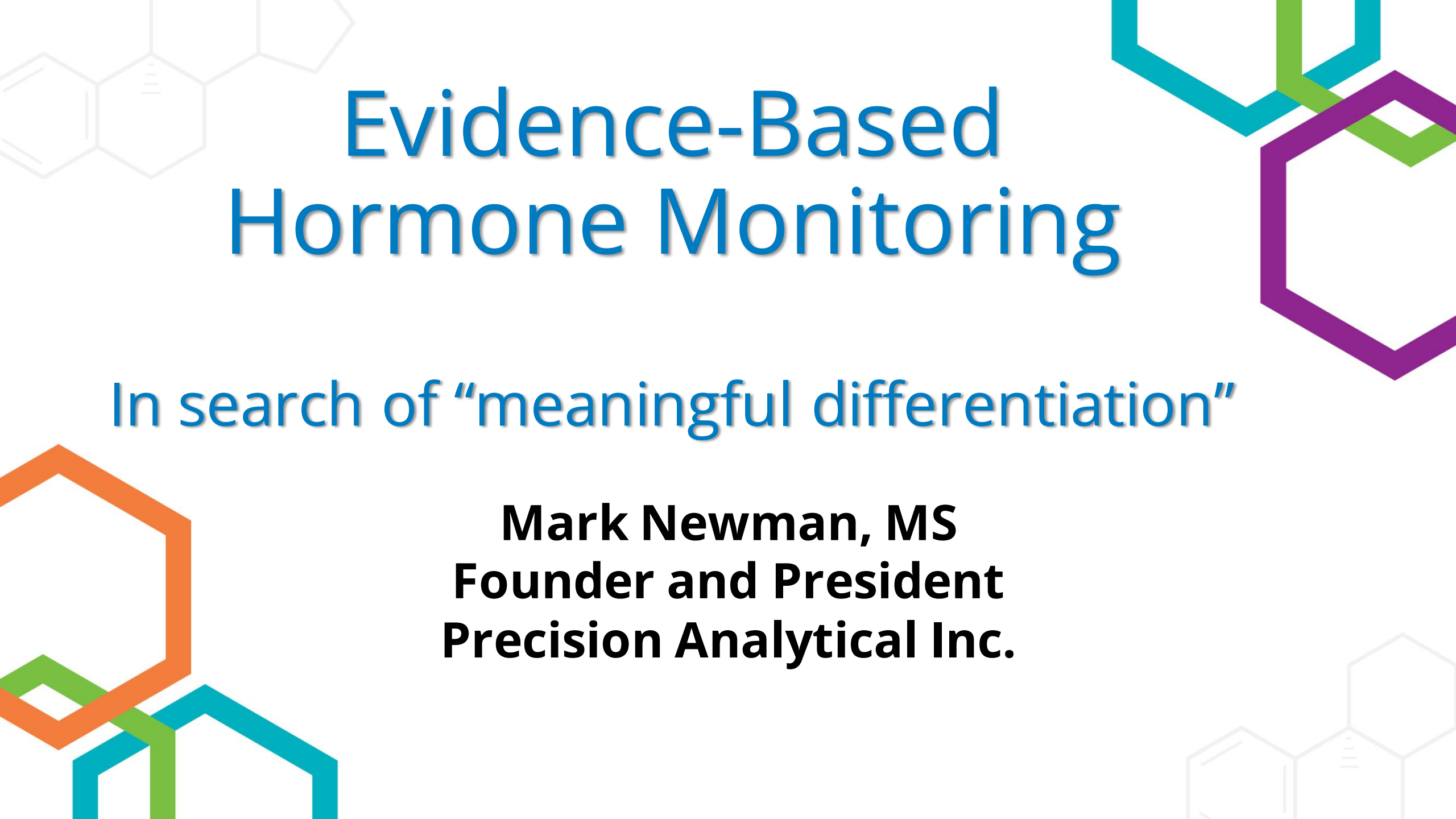
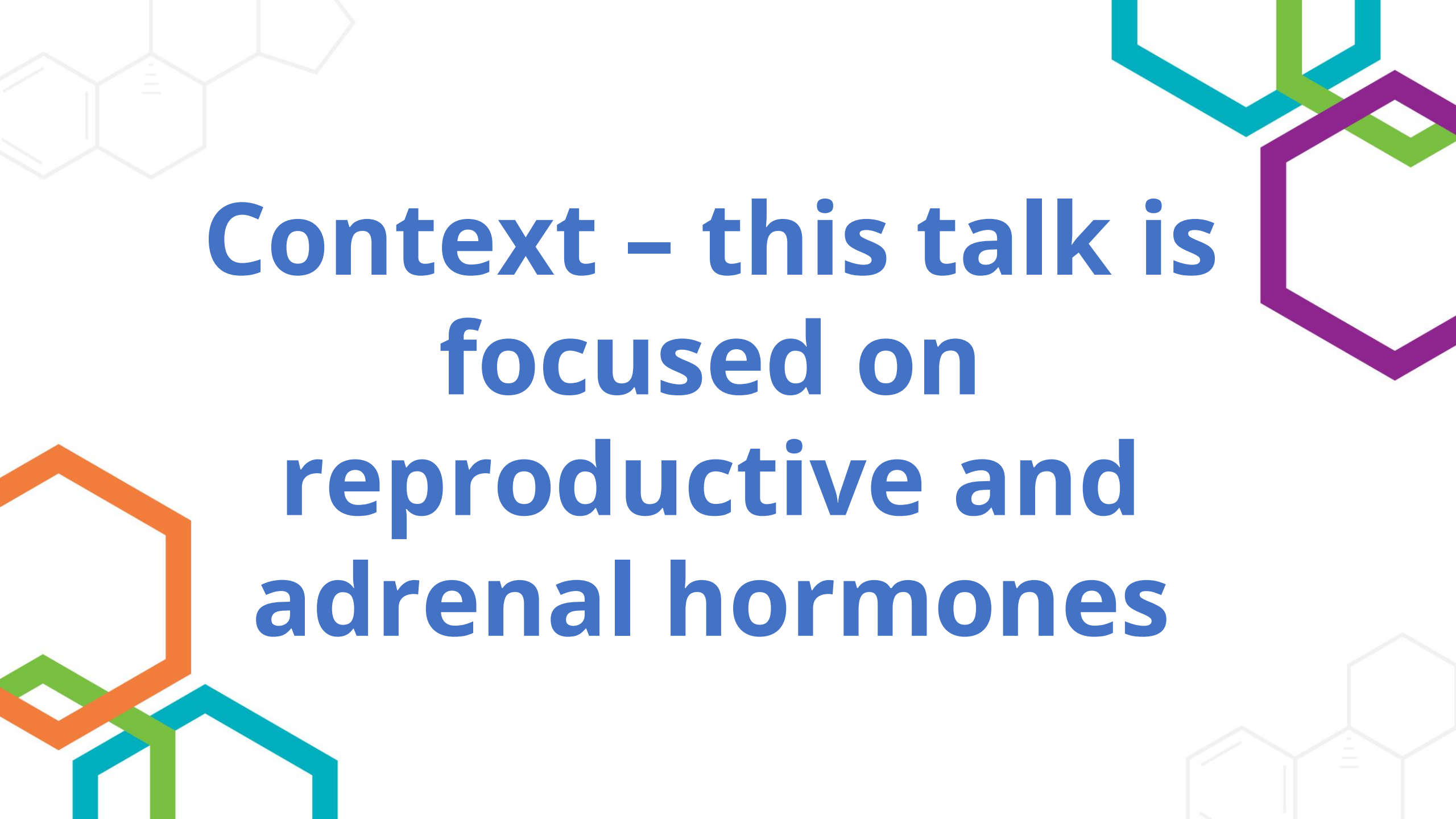


The slide features decorative hexagonal patterns in the corners. The top-left and bottom-right corners contain faint, light gray hexagonal outlines. The top-right and bottom-left corners contain bold, thick outlines of hexagons in teal, green, and purple, some of which are interlocking.

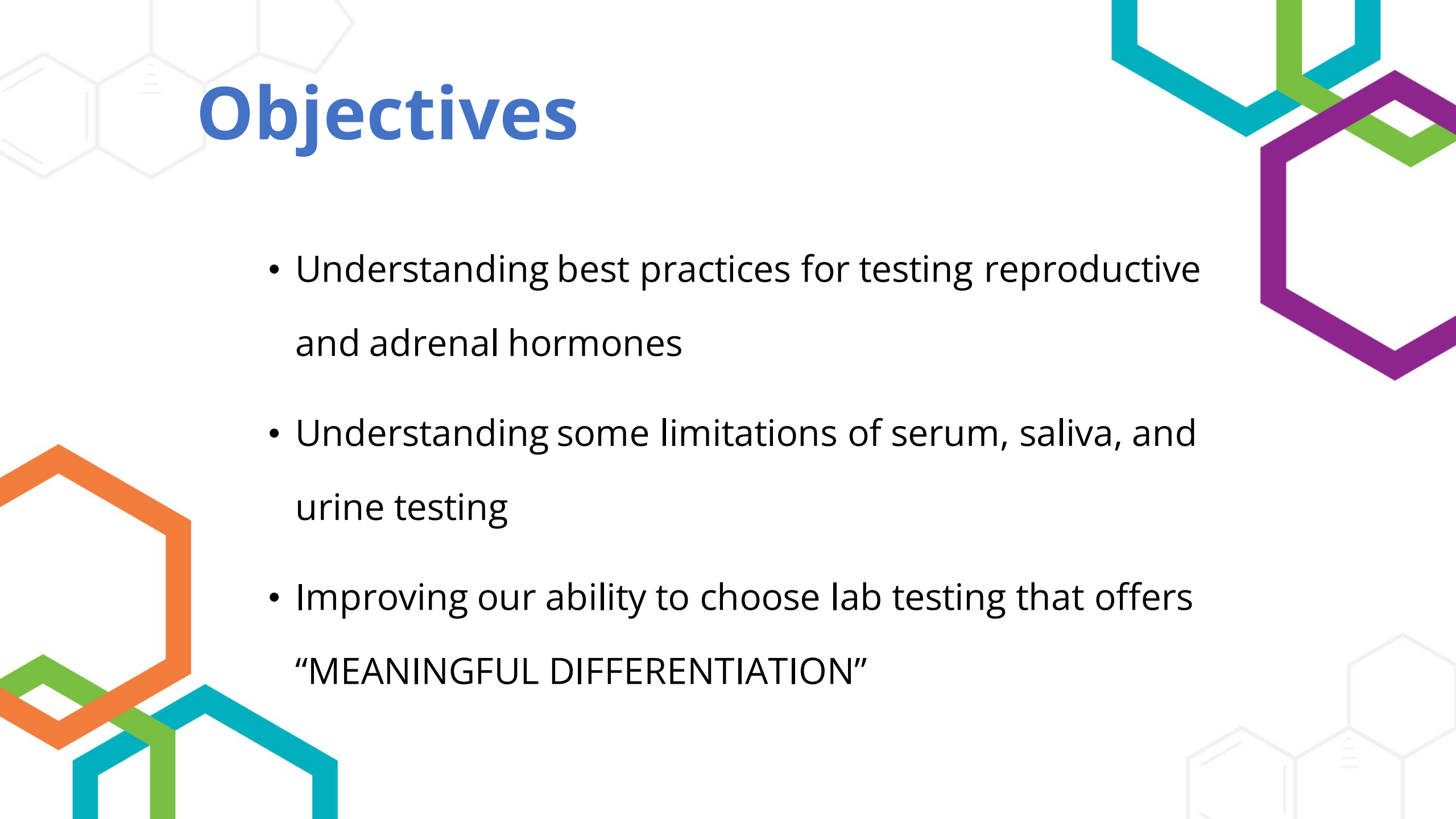
Evidence-Based Hormone Monitoring

In search of “meaningful differentiation”

Mark Newman, MS
Founder and President
Precision Analytical Inc.

The slide features decorative hexagonal patterns in the corners. The top-left and bottom-right corners contain faint, light gray outlines of fused hexagons. The top-right and bottom-left corners contain thick, overlapping outlines of hexagons in teal, green, and purple. The text is centered in a bold, blue, sans-serif font.

**Context – this talk is
focused on
reproductive and
adrenal hormones**

The slide features decorative hexagonal patterns in the corners. The top-left corner has a light gray pattern. The top-right corner has a pattern with teal, green, and purple lines. The bottom-left corner has a pattern with orange, green, and teal lines. The bottom-right corner has a light gray pattern.

Objectives

- Understanding best practices for testing reproductive and adrenal hormones
- Understanding some limitations of serum, saliva, and urine testing
- Improving our ability to choose lab testing that offers “MEANINGFUL DIFFERENTIATION”

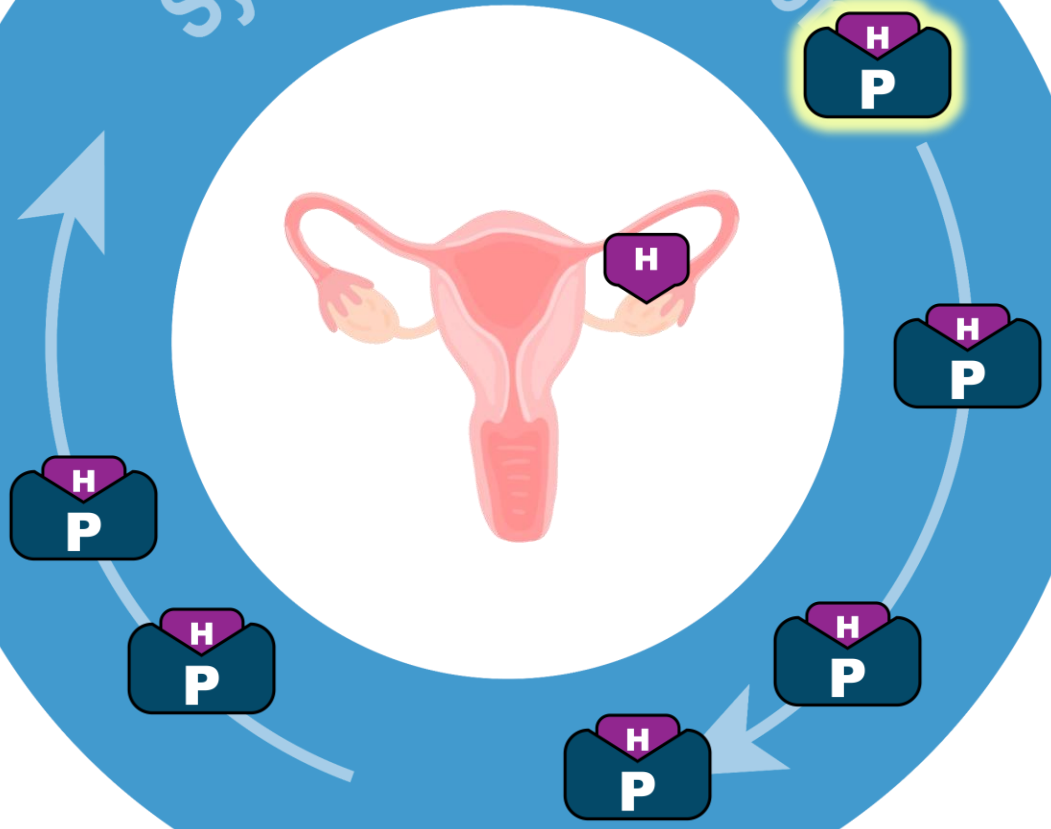
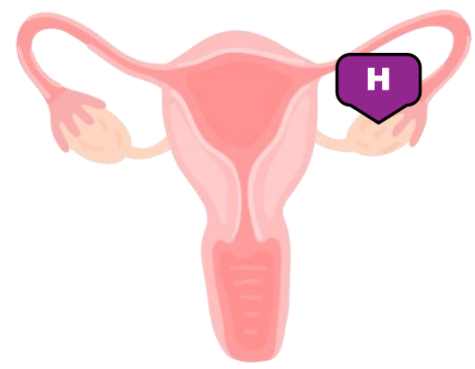


Serum, Saliva, Urine Testing?

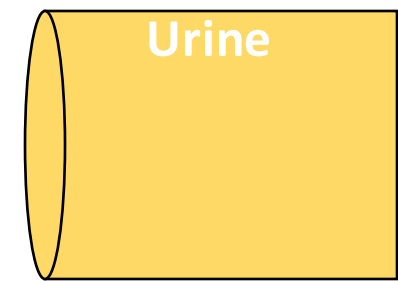
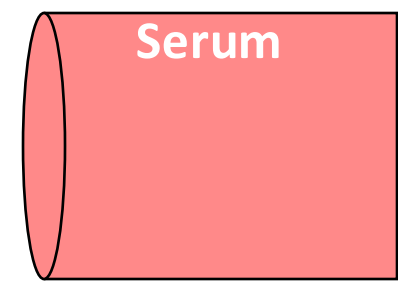
What's the Difference?

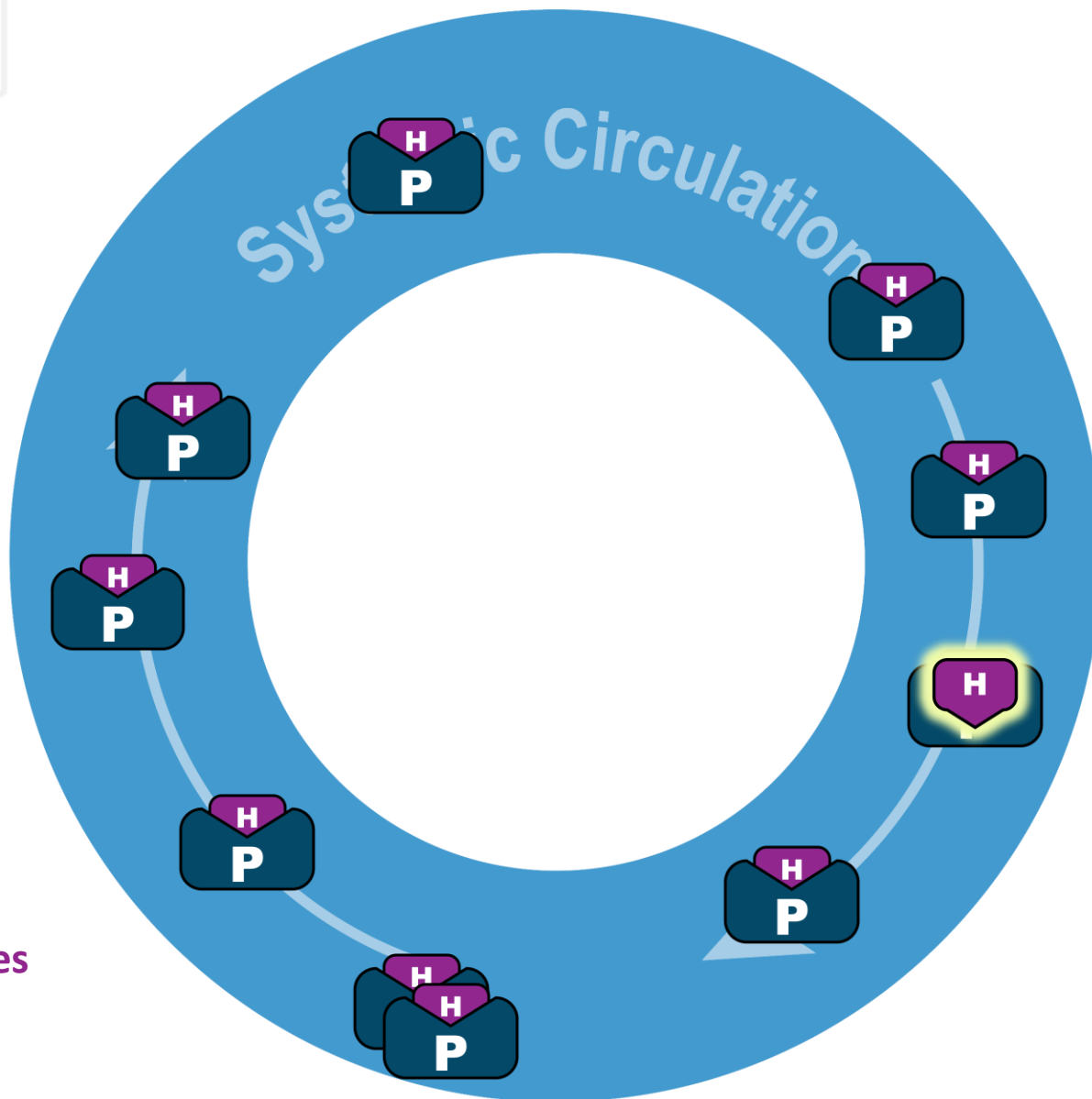


Systemic Circulation

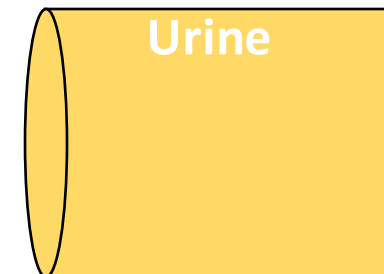
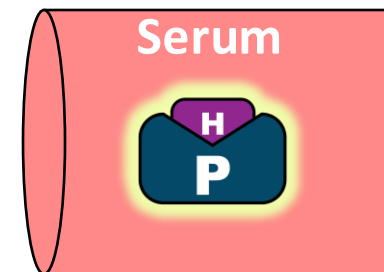


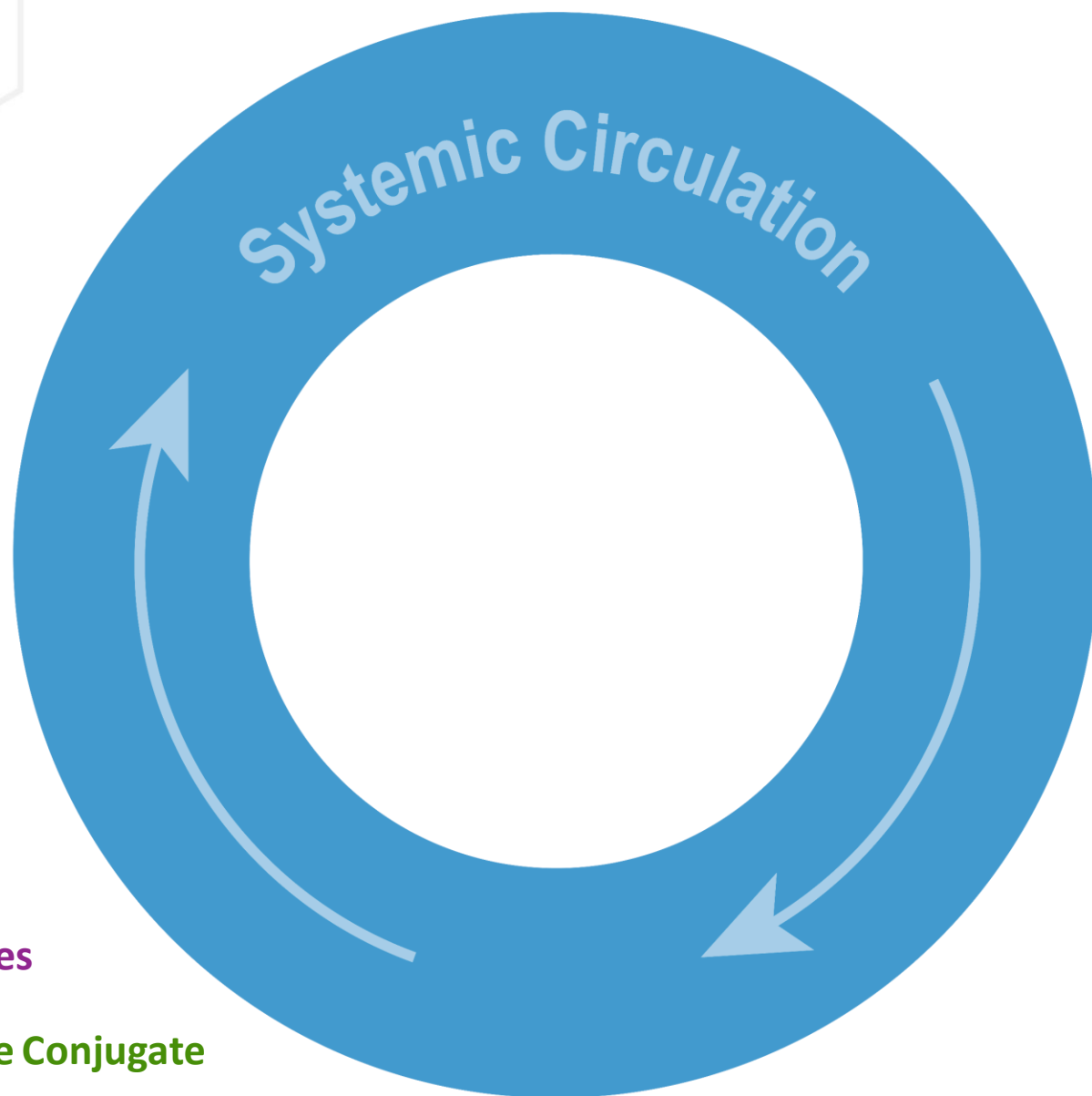
 Protein
 Hormones





 **Protein**
 **Hormones**





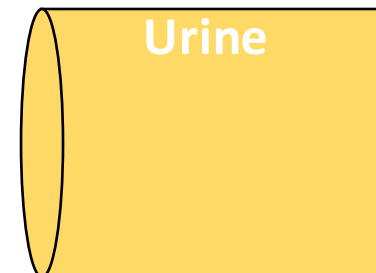
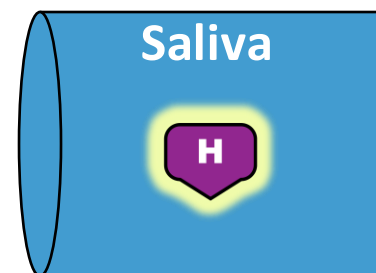
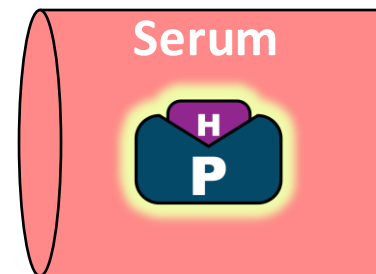
Protein



Hormones



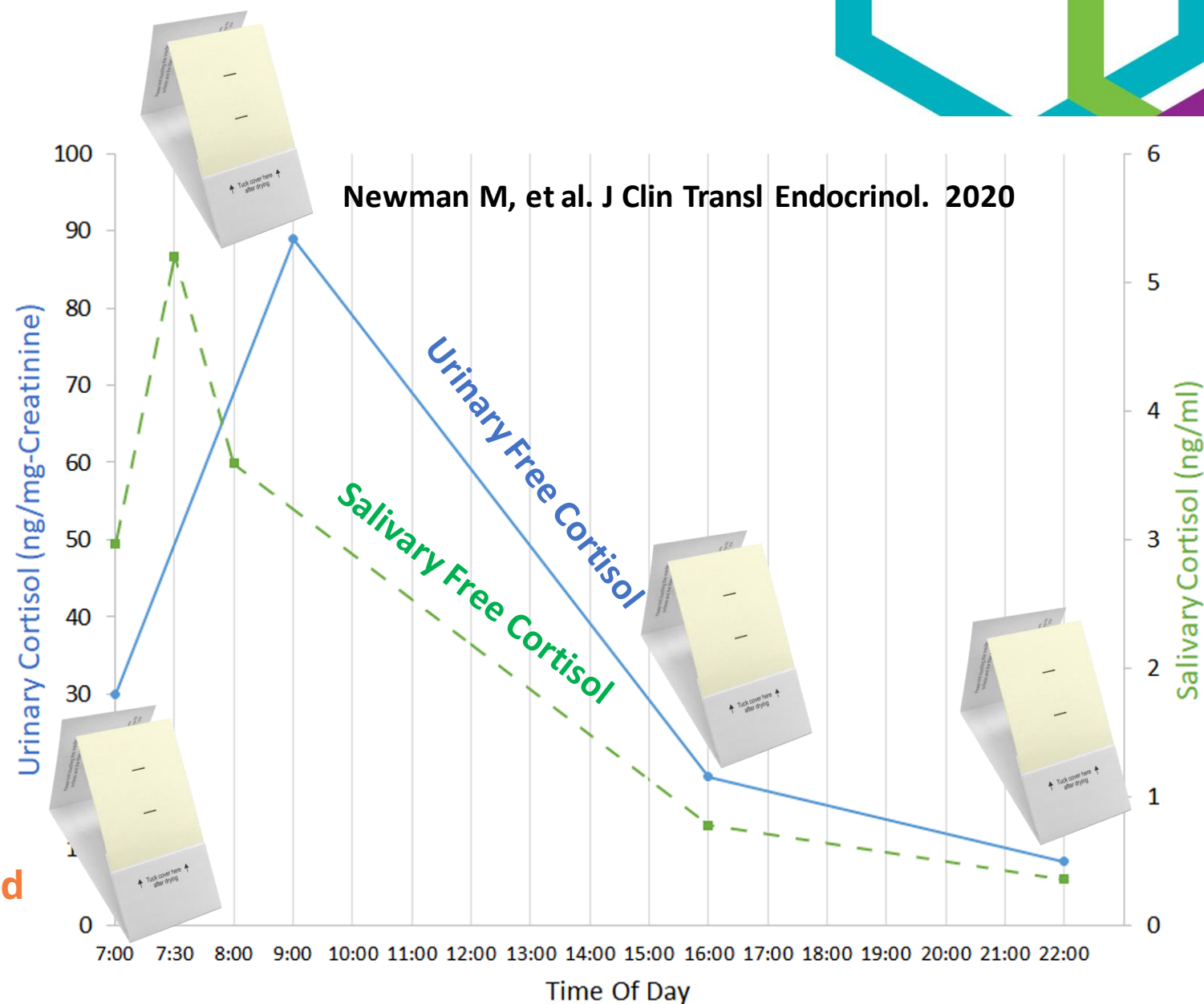
Hormone Conjugate

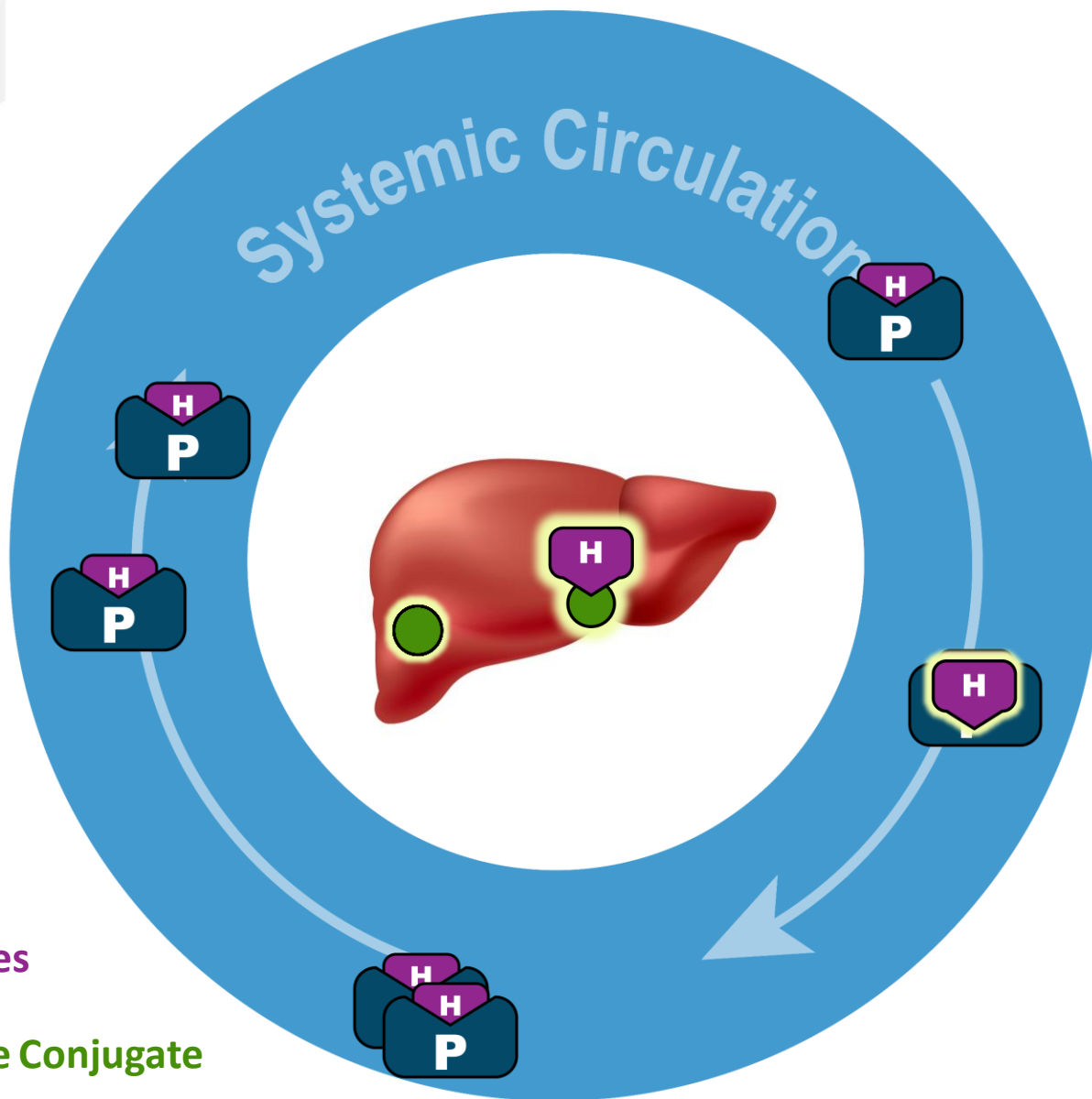


Dried Urine & Saliva Free Cortisol

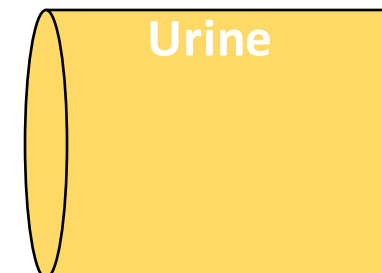
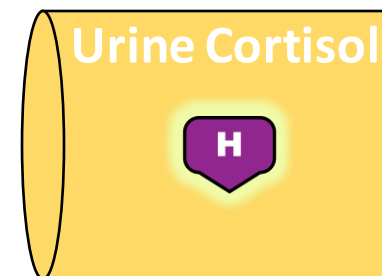
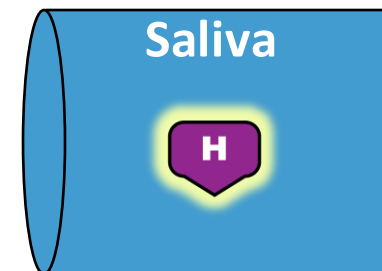
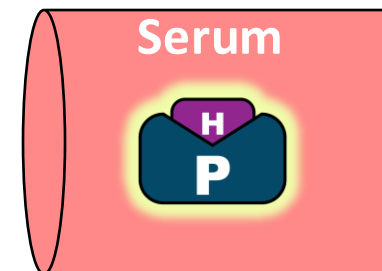
Precision
Analytical Data
N=68

Peer-Reviewed Published



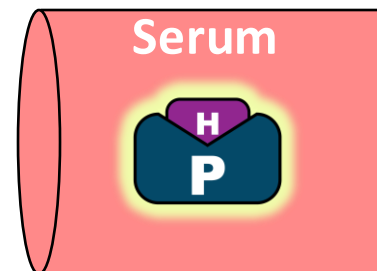


-  Protein
-  Hormones
-  Hormone Conjugate

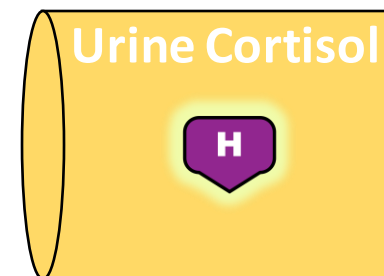
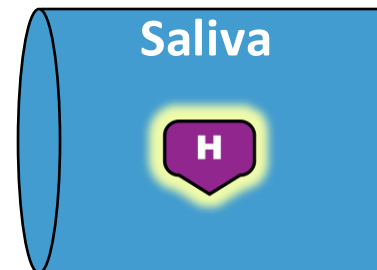


Serum, Saliva, or Urine?

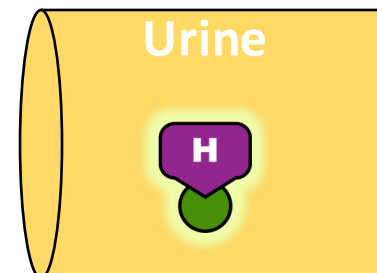
Protein-bound (SHBG, Albumin)



Free (unbound) Hormone



Hormone Conjugate (water-soluble)





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

Peer-Reviewed
Published
Validation

Serum correlations, etc.
for E2, Pg

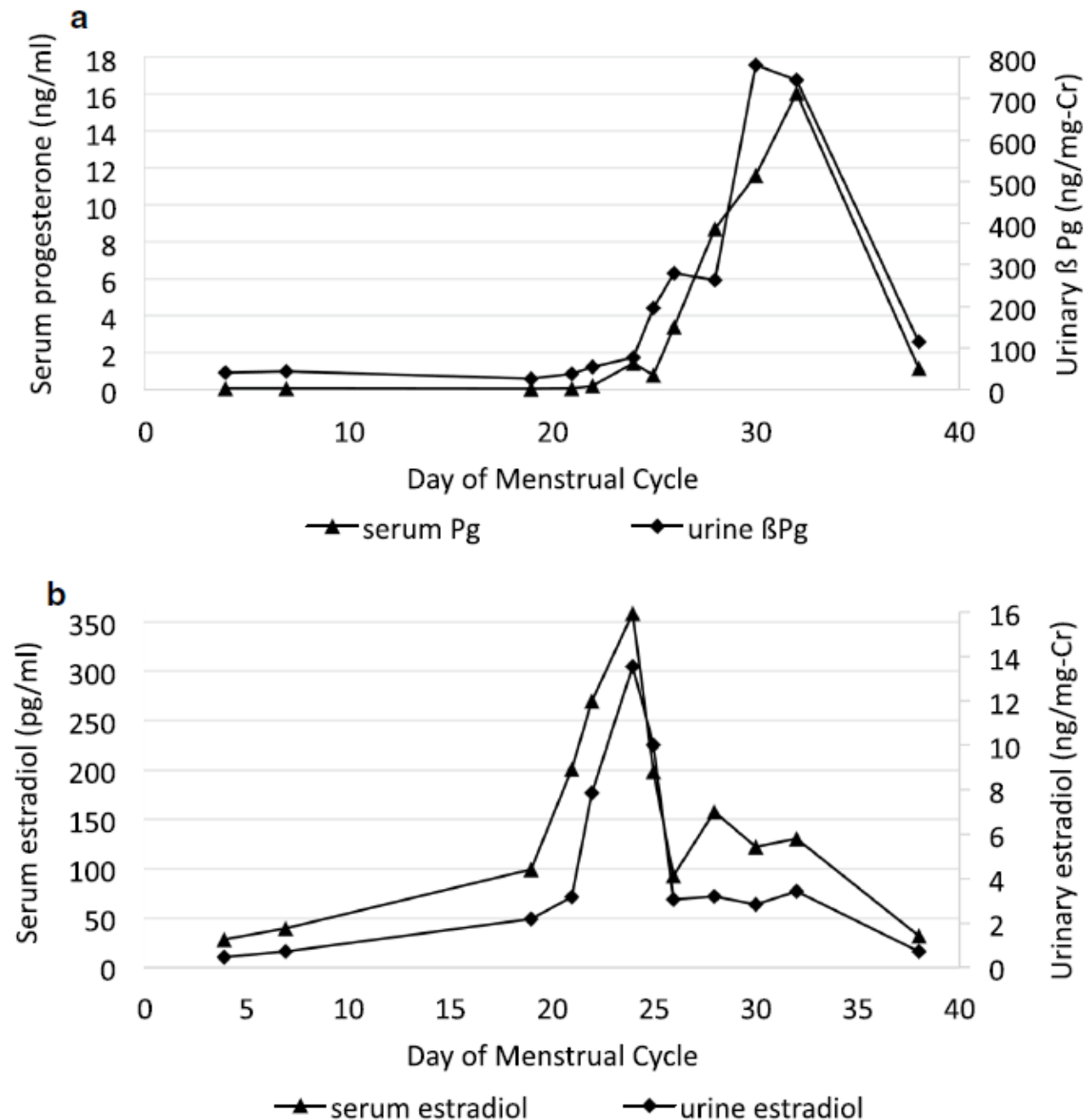


Fig. 2 Hormone profiles of serum progesterone versus urinary β-pregnenediol (a) and serum versus urinary estradiol (b) in one premenopausal woman's cycle. Metabolites of subject 2. Cr, creatinine; βPg, β-pregnenediol

Serum, Saliva, or Urine?



Dried Urine

- 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)

Serum

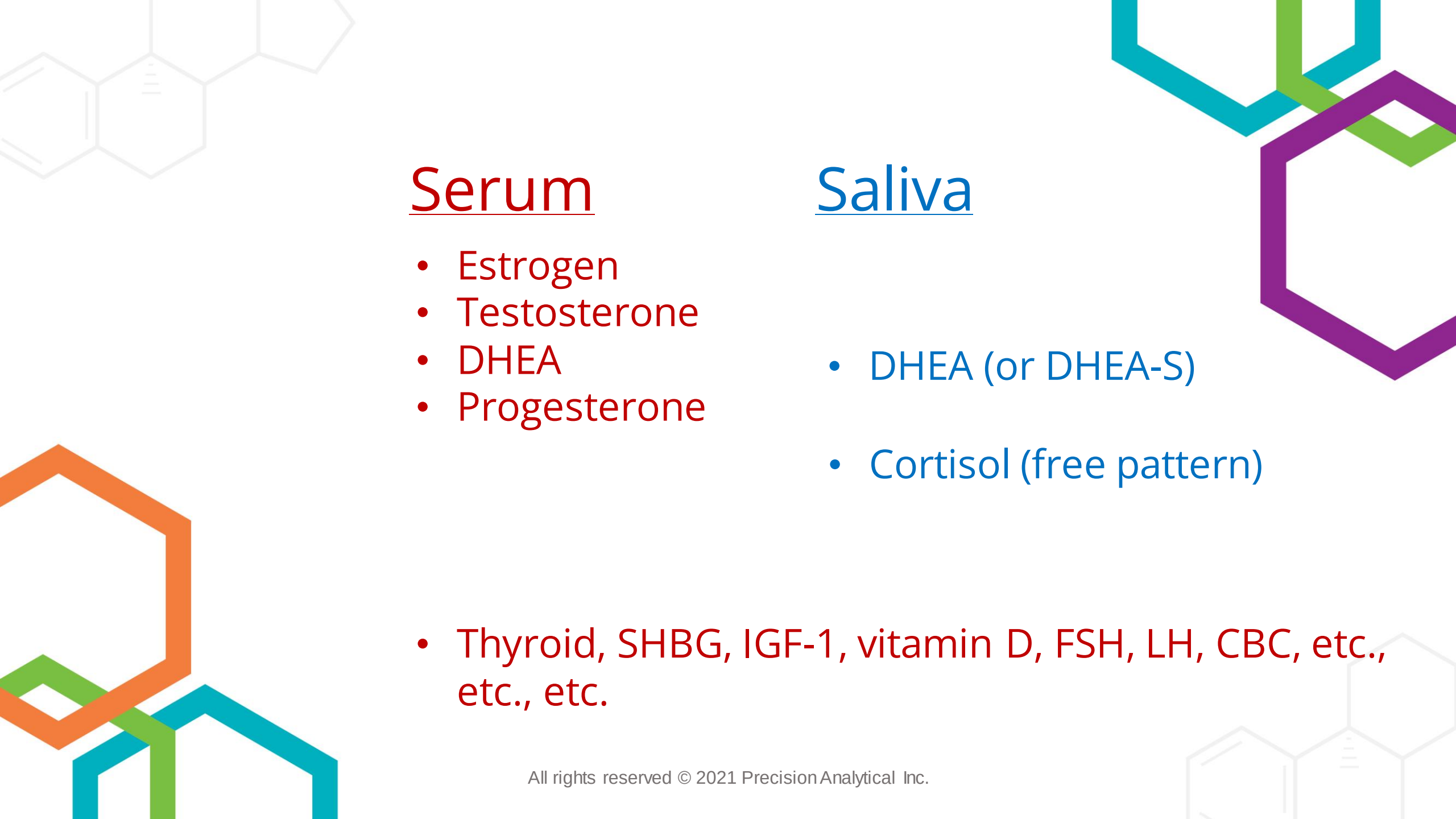
- Estrogen (production)
- Testosterone (production)
- DHEA (production)
- Progesterone (production)

Saliva

- Cortisol (free)

General Guideline for Hormone Testing

Serum is the default
Cortisol drives us to **saliva**
Comprehensiveness to **urine**



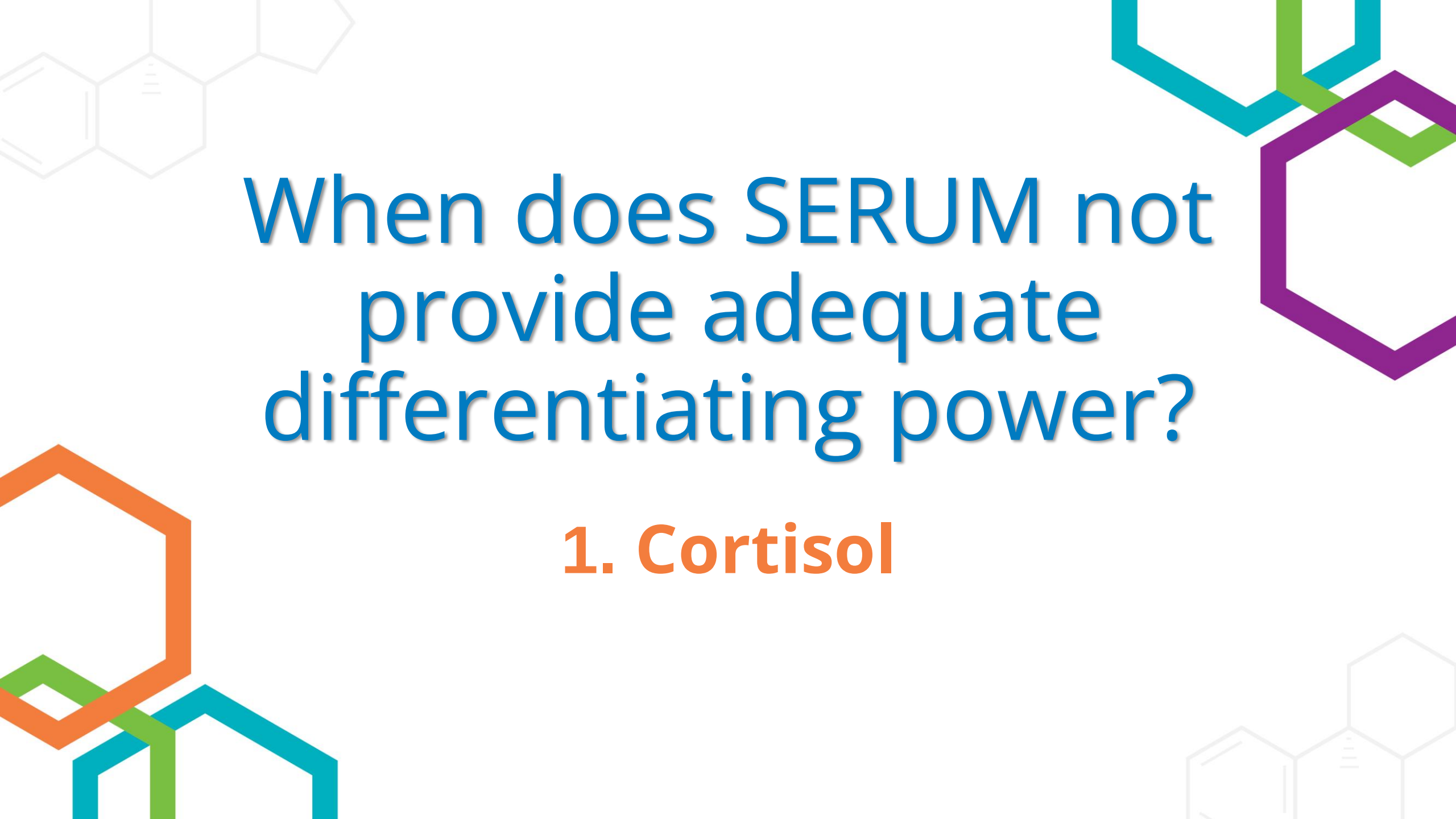
Serum

- Estrogen
- Testosterone
- DHEA
- Progesterone

Saliva

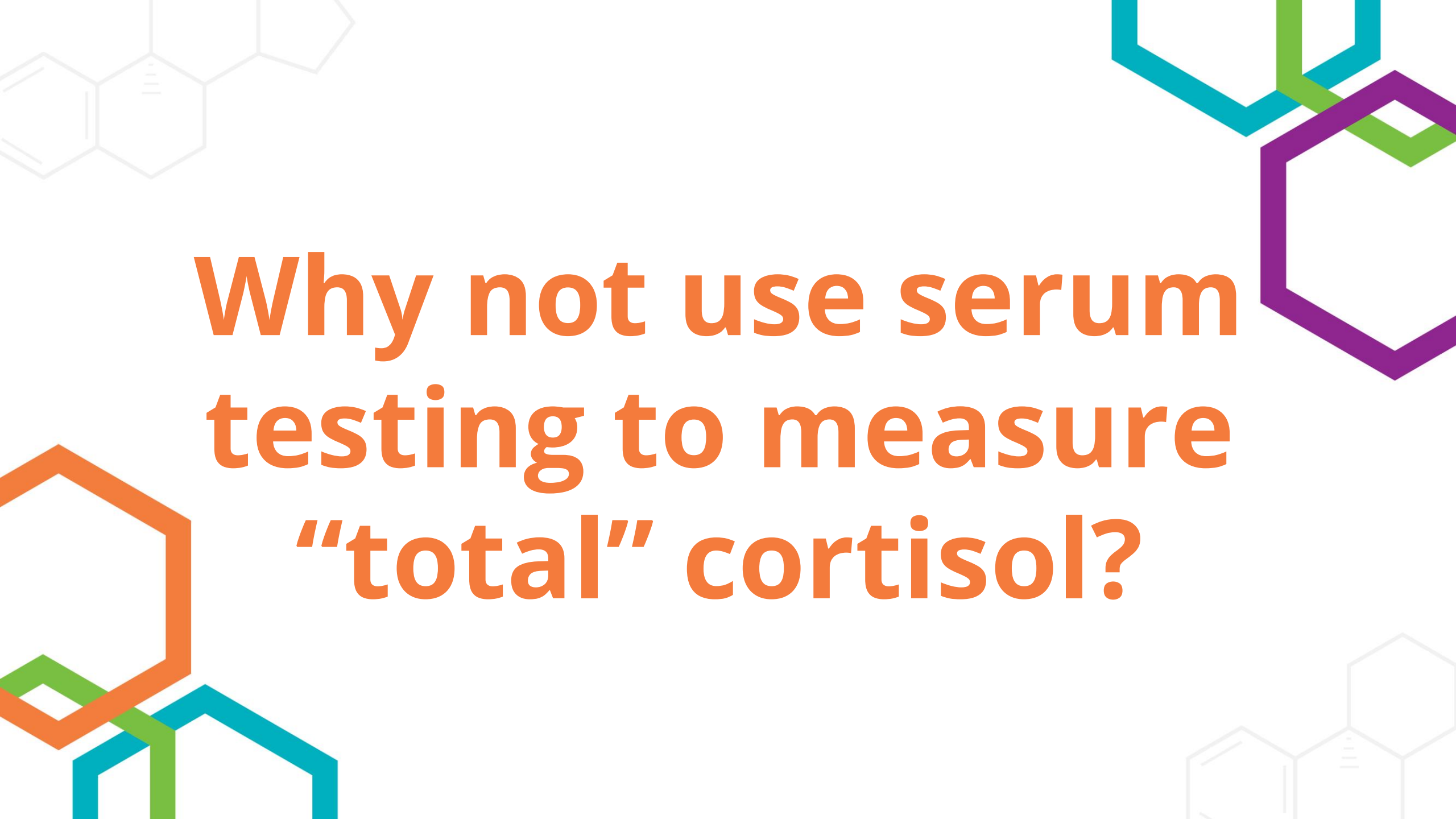
- DHEA (or DHEA-S)
- Cortisol (free pattern)

- Thyroid, SHBG, IGF-1, vitamin D, FSH, LH, CBC, etc., etc., etc.

The background features a light gray chemical structure of a steroid-like molecule in the top-left and bottom-right corners. Overlaid on this are several large, stylized hexagons in teal, green, orange, and purple, some of which are interconnected or overlapping.

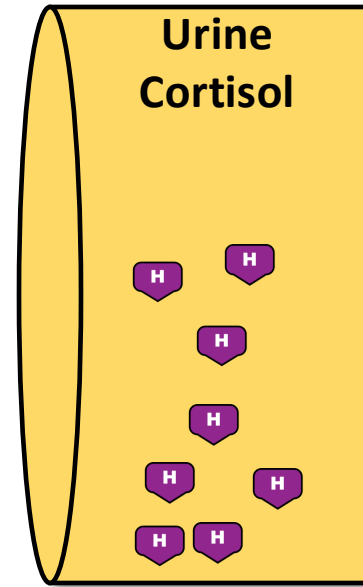
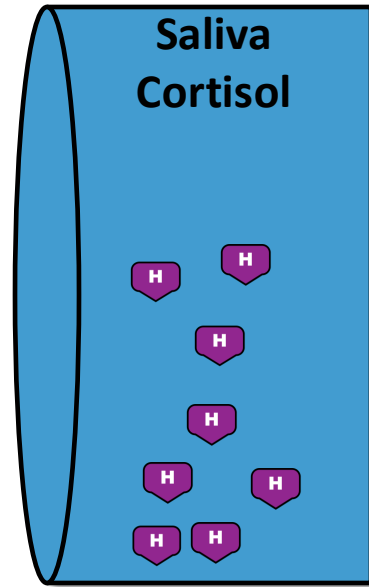
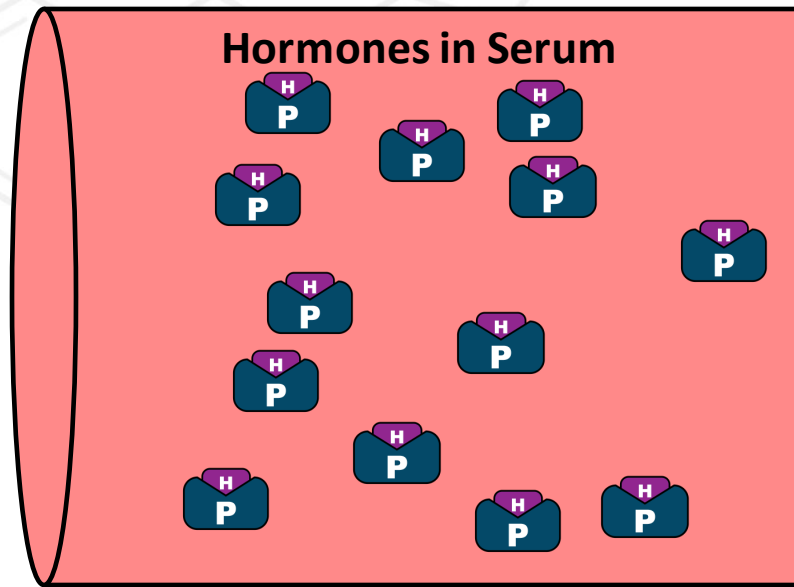
When does SERUM not
provide adequate
differentiating power?

1. Cortisol

The background features decorative hexagonal patterns in the corners. The top-left and bottom-right corners have faint, light gray outlines of hexagonal rings. The top-right and bottom-left corners have thick, solid-colored outlines of hexagonal rings in teal, green, and purple. The text is centered in the middle of the slide.

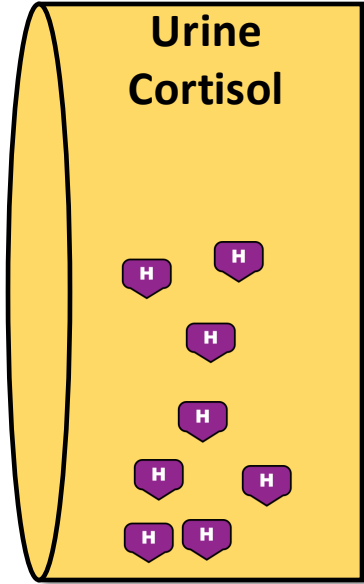
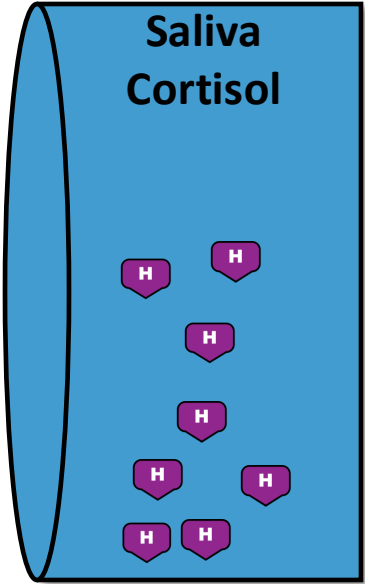
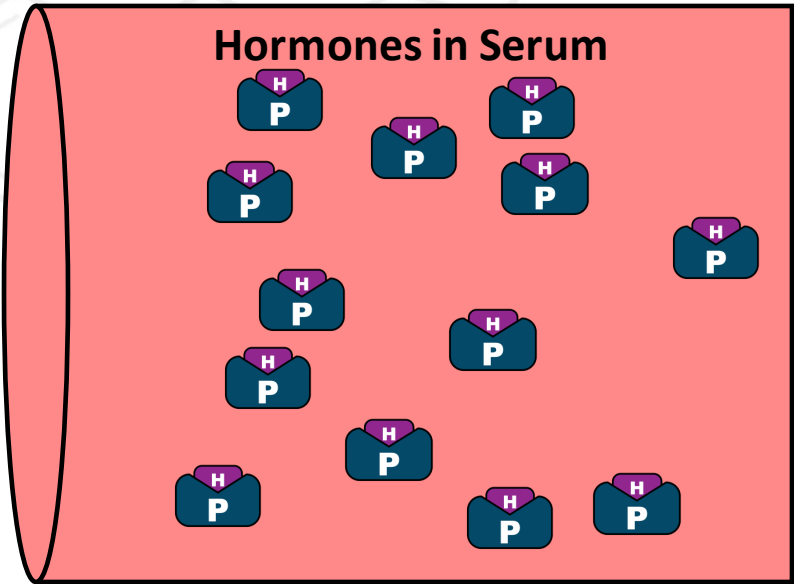
**Why not use serum
testing to measure
“total” cortisol?**

Patient NOT on Birth Control



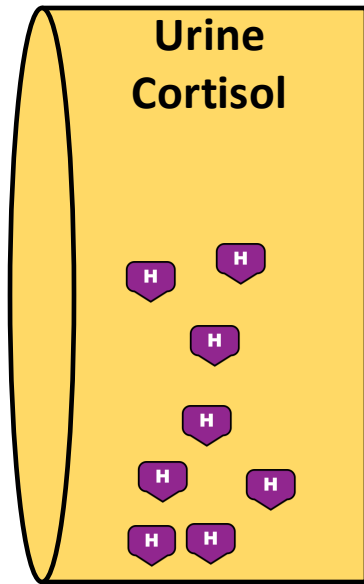
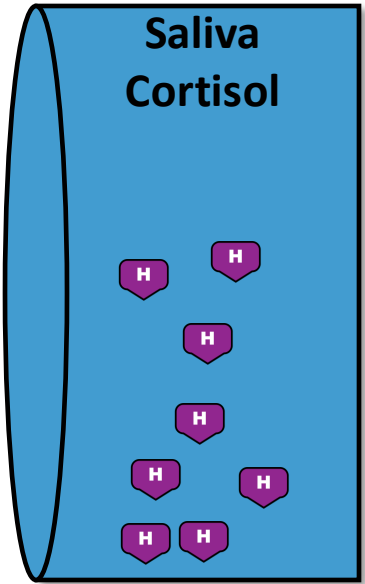
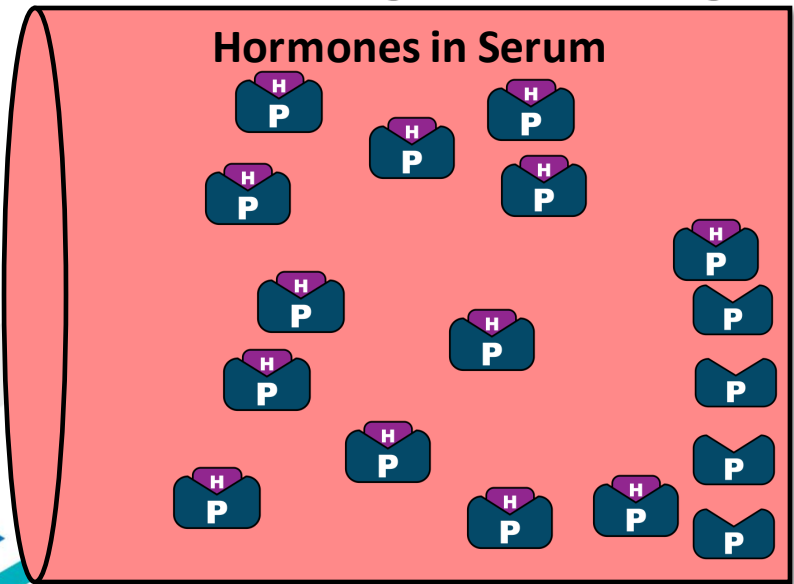
 **Protein**
 **Hormones**

Patient NOT on Birth Control



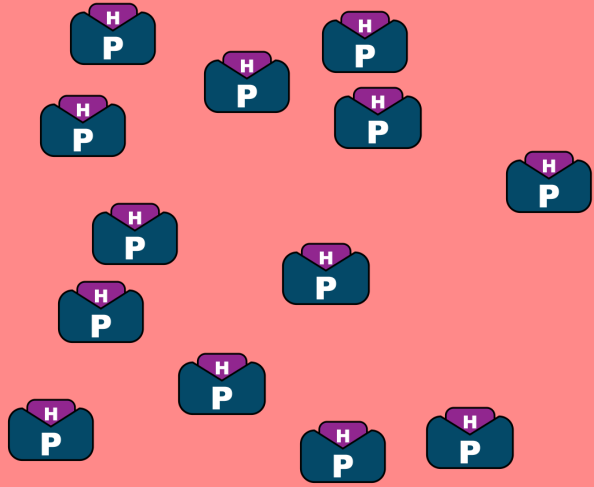
 **Protein**
 **Hormones**

Patient begins taking Birth Control



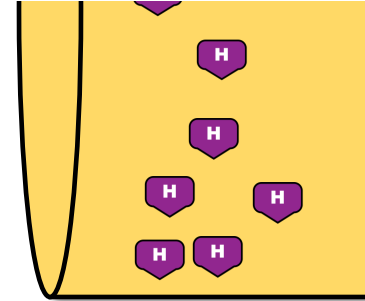
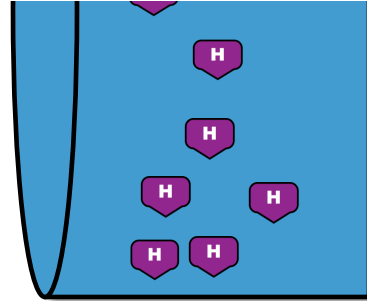
Patient NOT on Birth Control

Hormones in Serum



Physiol. Res. 57 (Suppl. 1): S193-S199, 2008

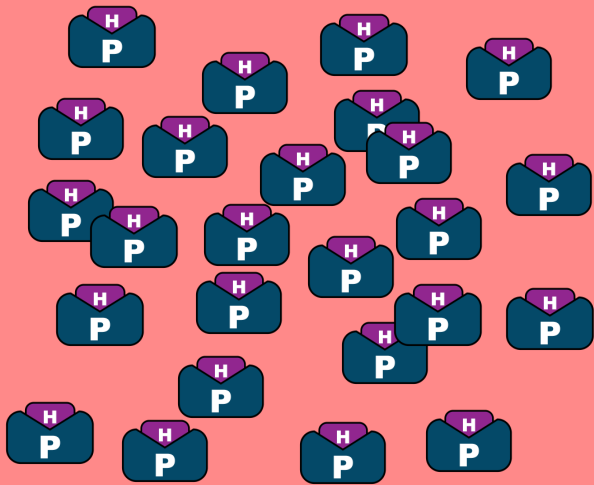
Comparison of Total and Salivary Cortisol in a Low-Dose ACTH (Synacthen) Test: Influence of Three-Month Oral Contraceptives Administration to Healthy Women



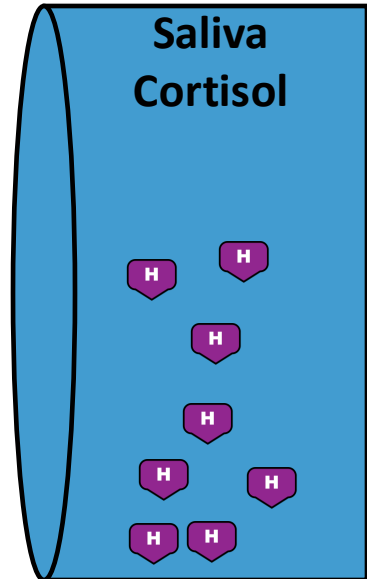
 **Protein**
 **Hormones**

Patient on Birth Control (no change in stress or stress response)

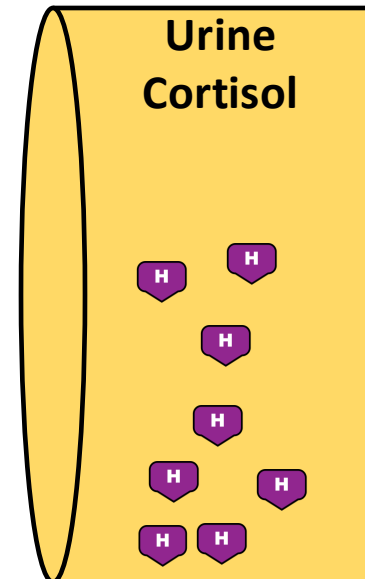
Hormones in Serum



Saliva Cortisol



Urine Cortisol



Cortisol Assessment

- Must be measured as “free” cortisol
- Diurnal pattern should be monitored
- Saliva is the gold standard
- Dried Urine is a viable option to measure diurnal free cortisol

Dried Urine

- Estrogen (production)
- Testosterone (production)
- DHEA (production)
- Progesterone (production)
- Cortisol (free pattern)

Serum

- Estrogen (production)
- Testosterone (production)
- DHEA (production)
- Progesterone (production)

Saliva

- Cortisol (free pattern)

Newman and Curran *BMC Chemistry* (2021) 15:18
<https://doi.org/10.1186/s13065-021-00744-3>

BMC Chemistry

RESEARCH ARTICLE

Open Access

Reliability of a dried urine test for comprehensive assessment of urine hormones and metabolites

Mark Newman* and Desmond A. Curran

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Dried Urine

- 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)

Serum

- Estrogen (production)
- Testosterone (production)
- DHEA (production)
- Progesterone (production)

Saliva

- Cortisol (free pattern)

DUTCH Complete

Dried Urine

- 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)

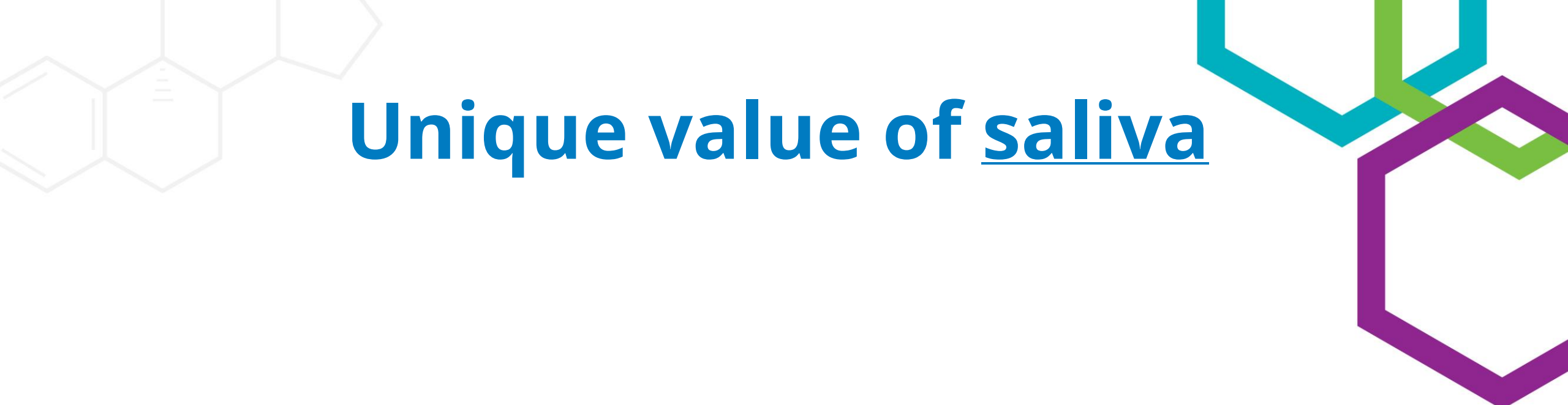
DUTCH Complete

Serum

- Estrogen (production)
- Testosterone (production)
- DHEA (production)
- Progesterone (production)

Saliva

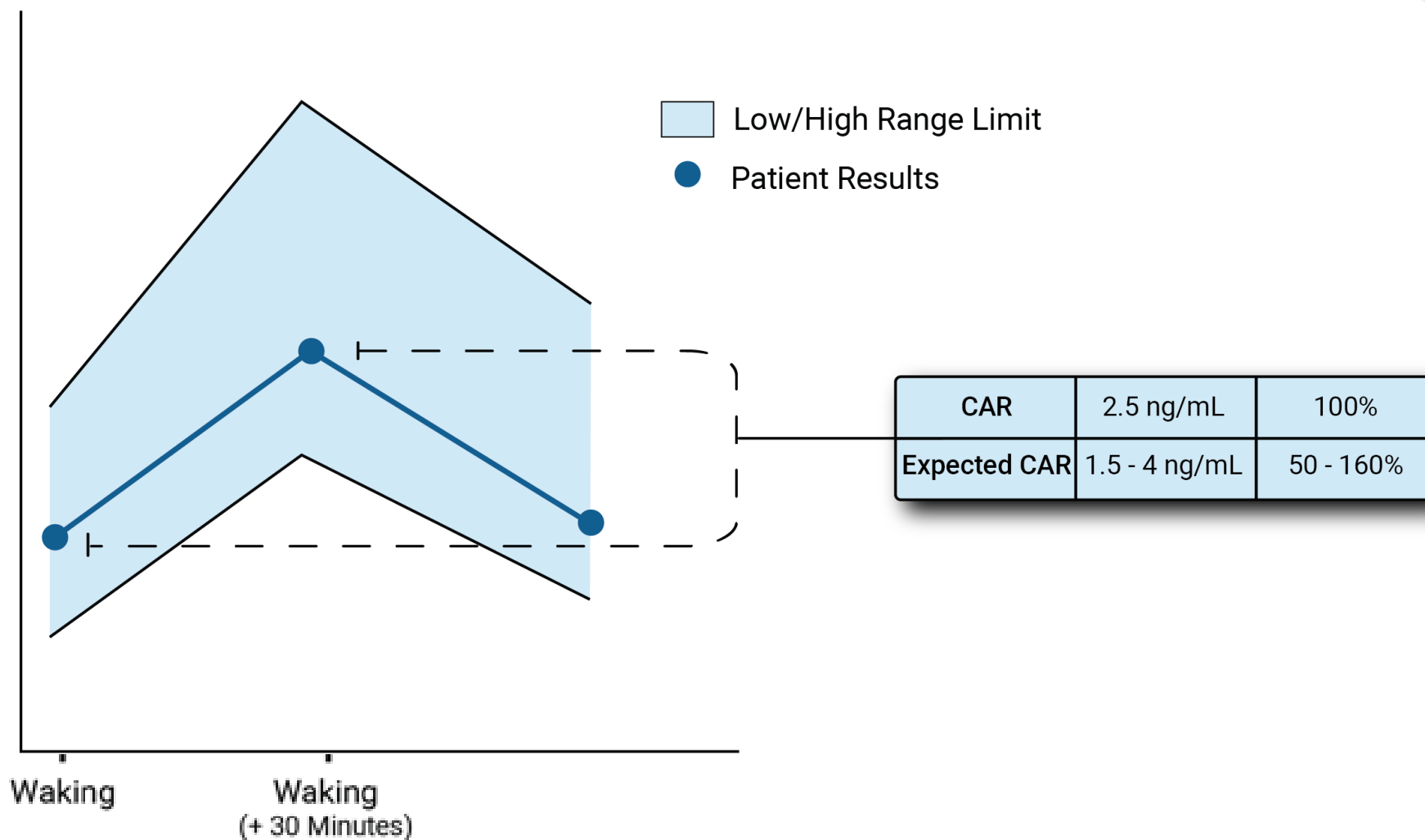
- Cortisol (free pattern)
- **Cortisol (CAR)**



Unique value of saliva

Cortisol Awakening Response (CAR)

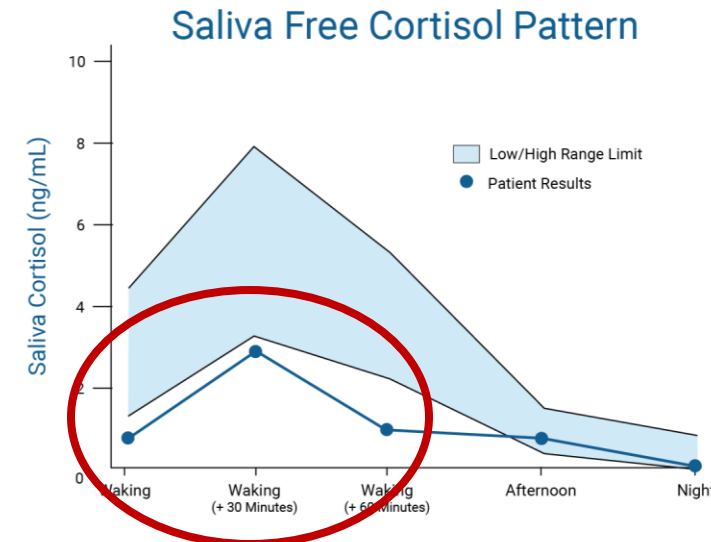
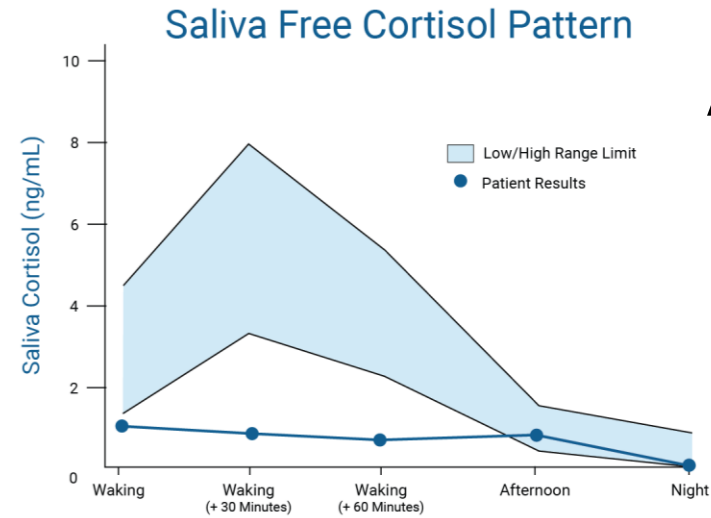
Cortisol Awakening Response



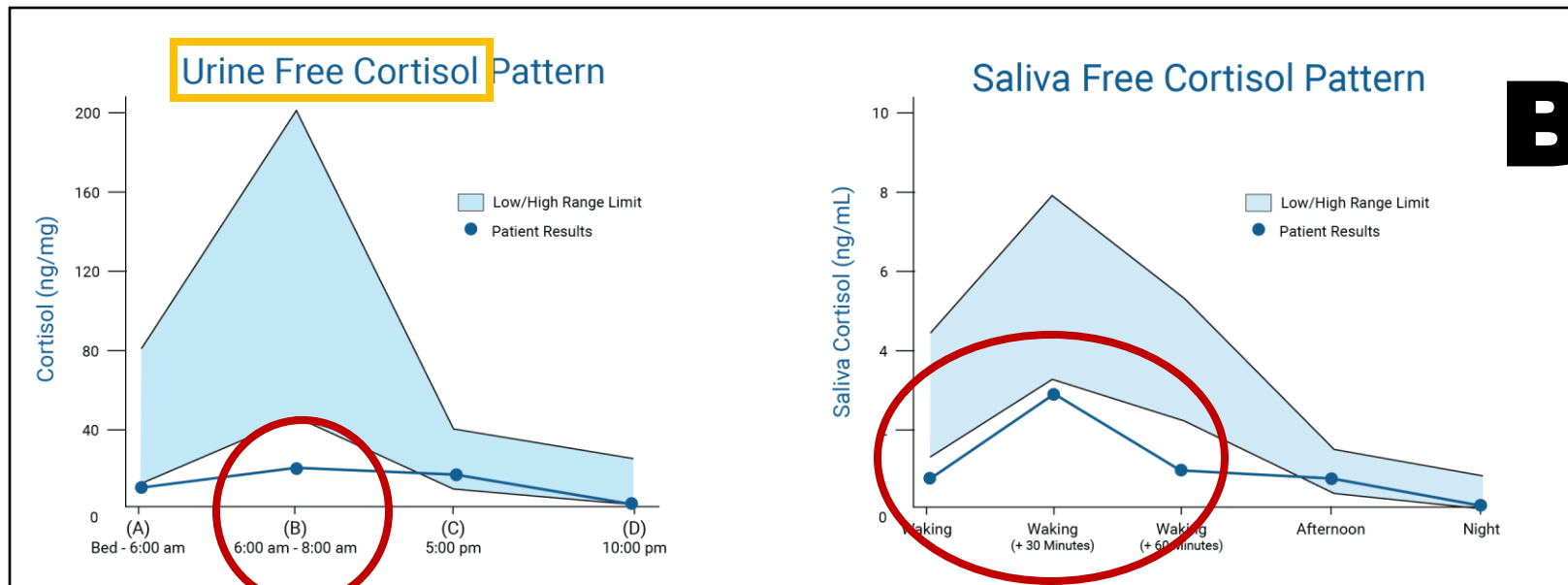
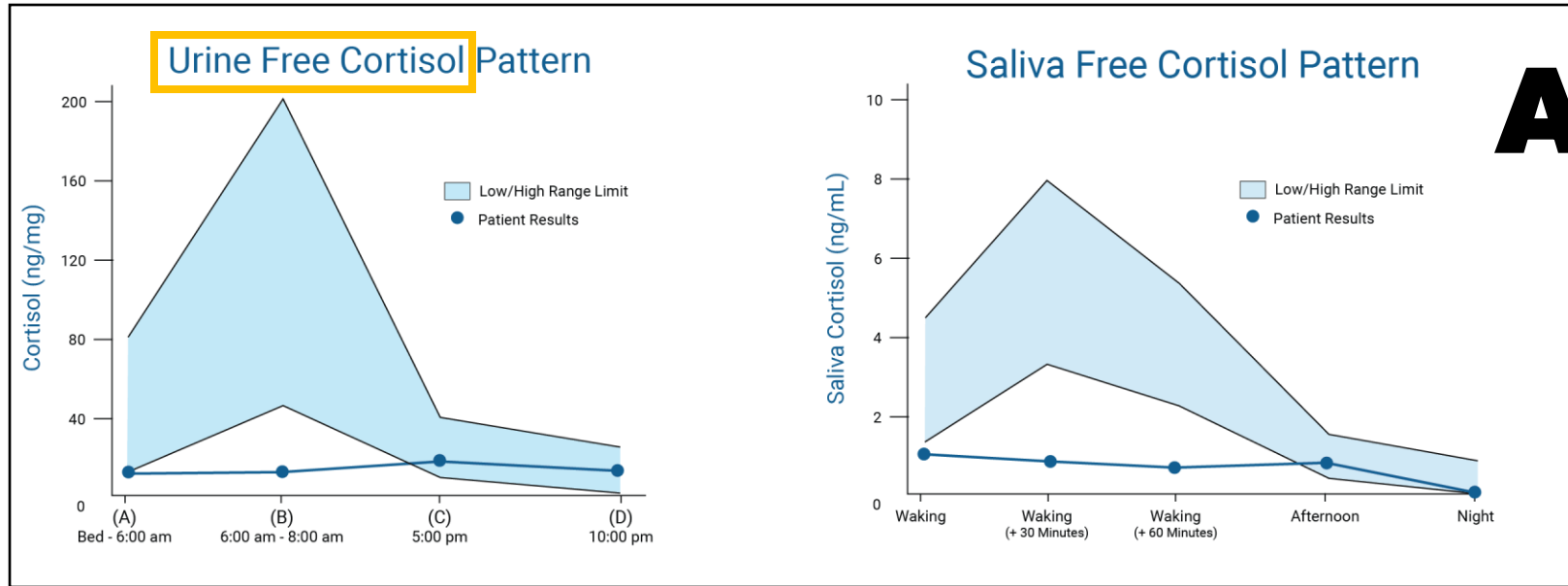
Benefit of the CAR

Patient B may have
more HPA Axis resiliency
compared to patient A

Patient B is below the reference
range but are still able to get a
rise in cortisol



Benefit of the CAR

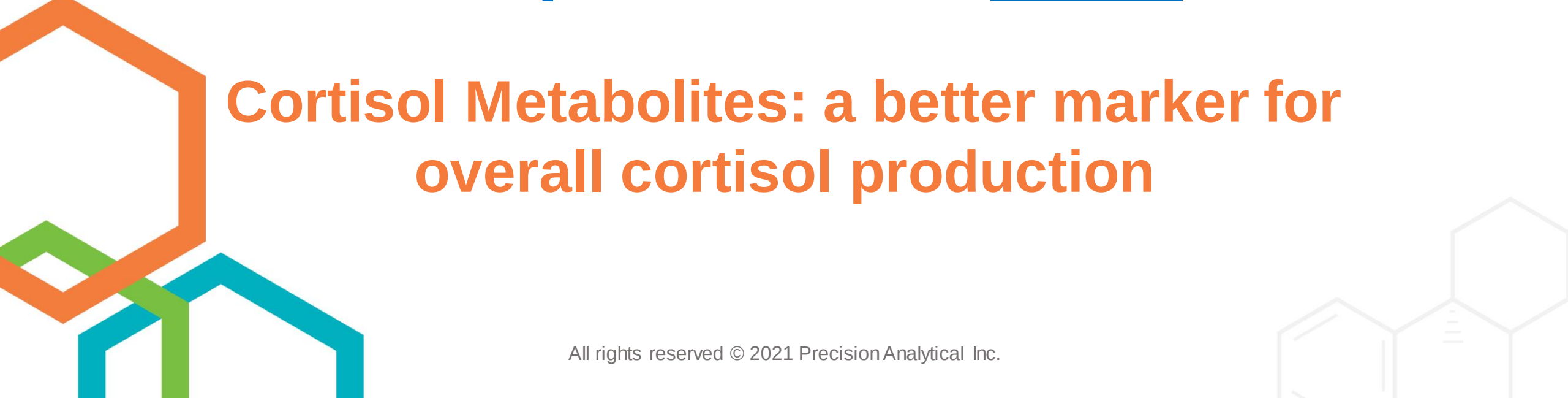




Unique value of saliva

Cortisol Awakening Response (CAR)

Unique value of urine



Cortisol Metabolites: a better marker for overall cortisol production

Dried Urine

- 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)

DUTCH Complete

Serum

- Estrogen (production)
- Testosterone (production)
- DHEA (production)
- Progesterone (production)

DUTCH Plus

Saliva

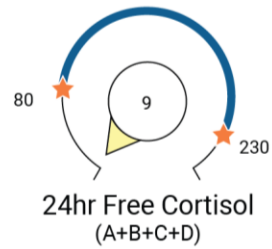
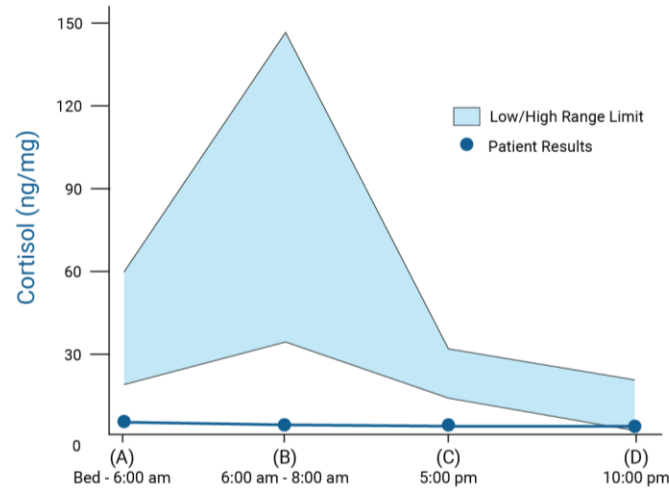
- Cortisol (free pattern)
- Cortisol (CAR)

The background features decorative hexagonal patterns in the corners. The top-left and bottom-right corners have faint, light gray hexagonal outlines. The top-right and bottom-left corners have thick, colorful hexagonal outlines in teal, green, and purple. The main text is centered in a bold, blue font.

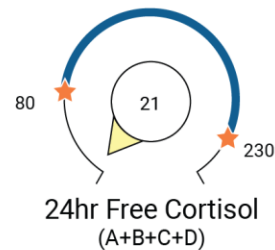
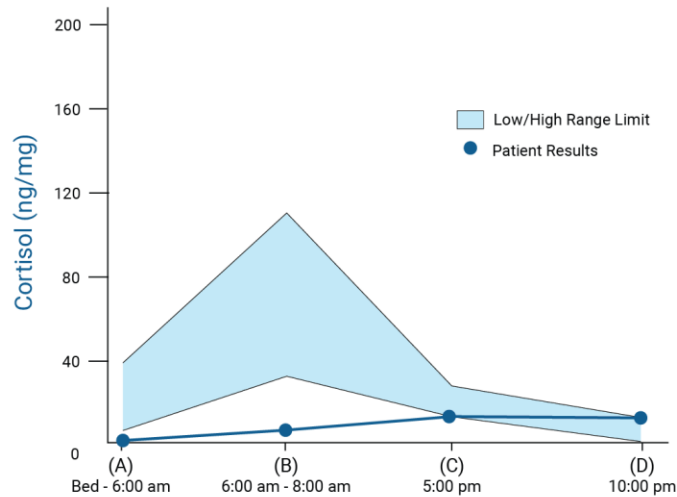
Why Measure Cortisol Metabolites?

Low Cortisol?

Urine Free Cortisol Pattern



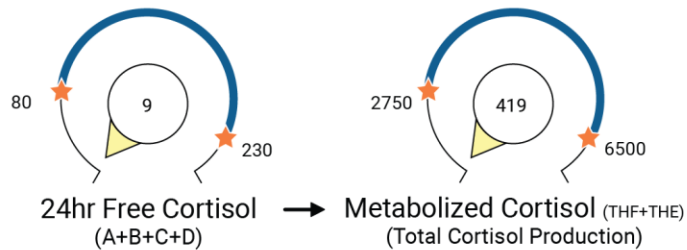
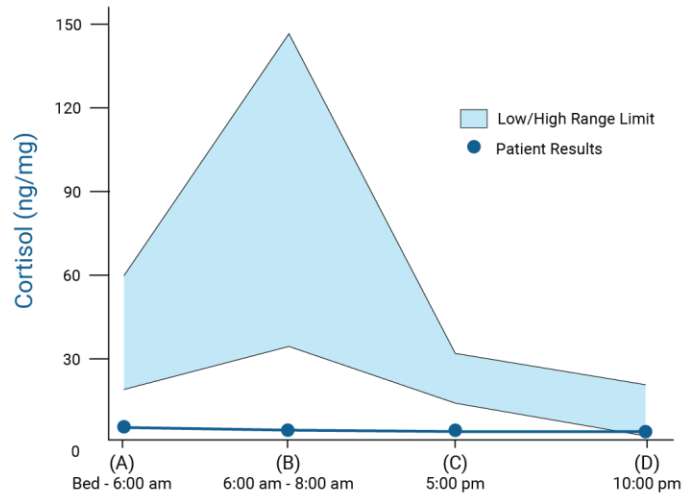
Urine Free Cortisol Pattern



**Prednisone
Suppression**

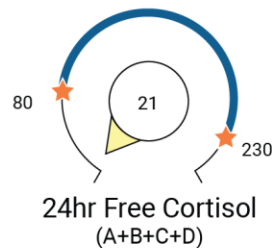
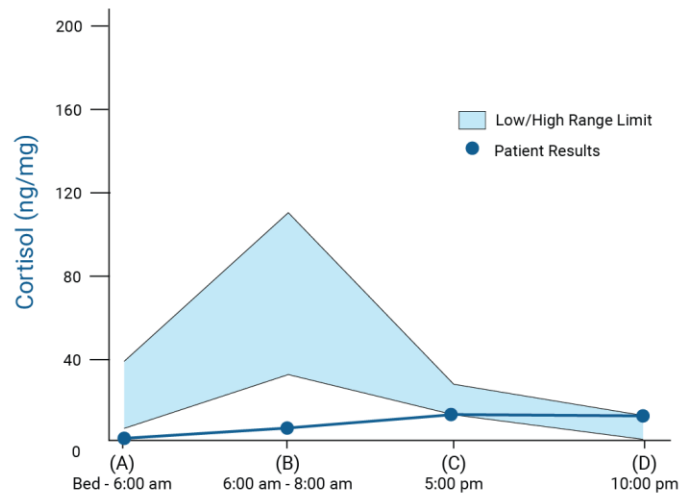
Low Cortisol?

Urine Free Cortisol Pattern



**Prednisone
Suppression**

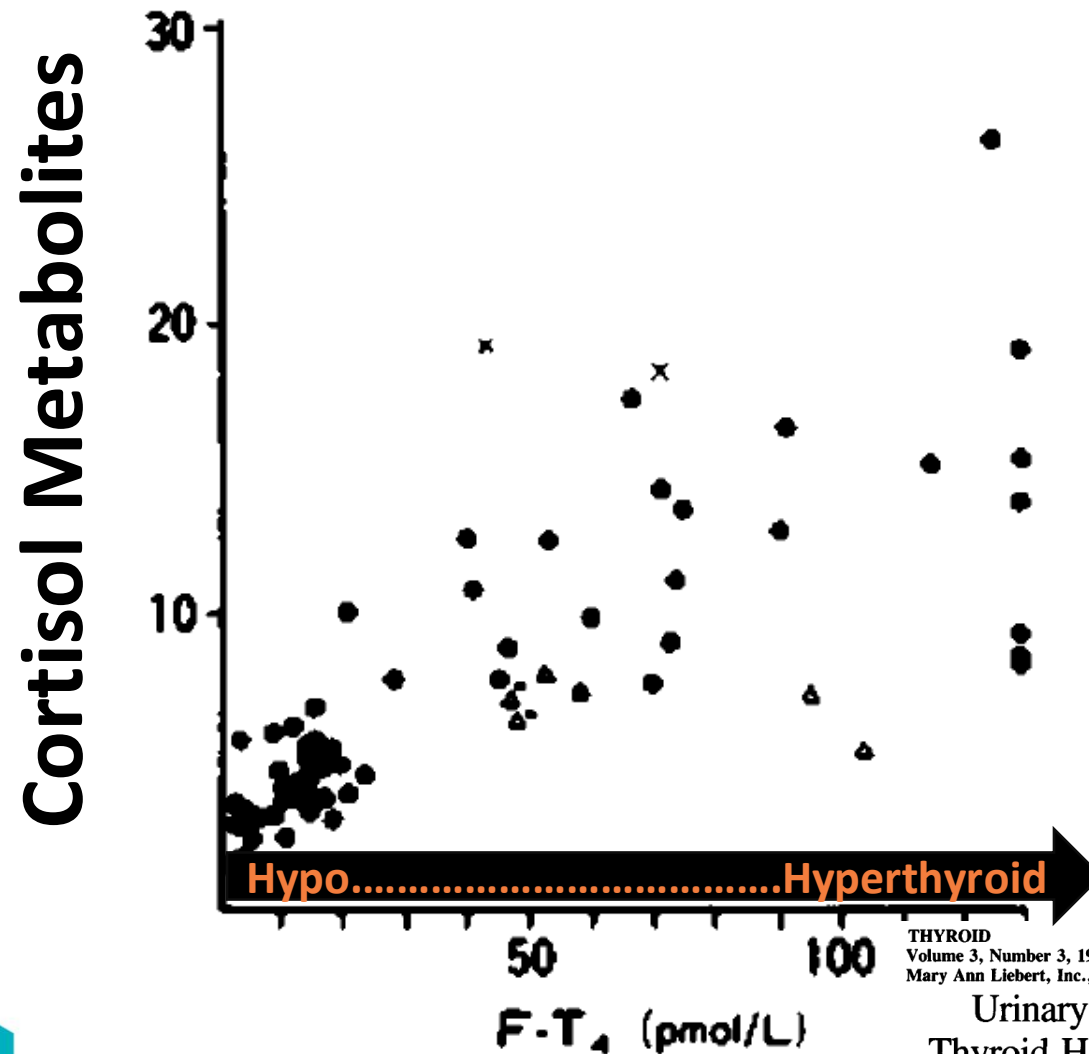
Urine Free Cortisol Pattern



Thyroid Overdose

- **fT3, T4 High**
- **Low TSH**

Thyroid directly impacts cortisol clearance

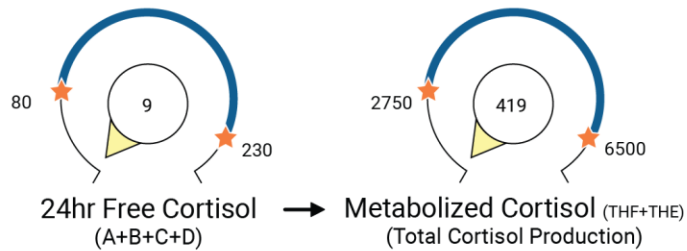
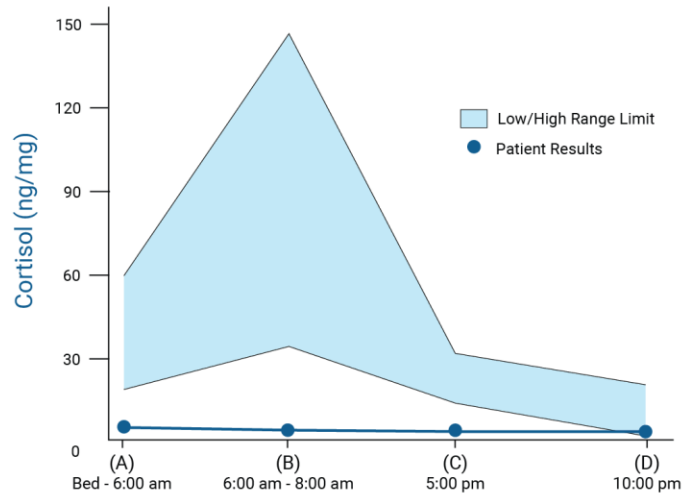


THYROID
Volume 3, Number 3, 1993
Mary Ann Liebert, Inc., Publishers

Urinary Cortisol Metabolites in the Assessment of Peripheral
Thyroid Hormone Action: Application for Diagnosis of Resistance
to Thyroid Hormone

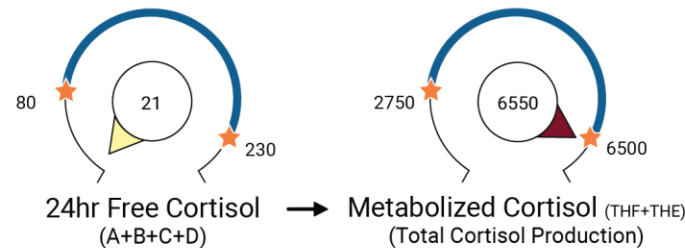
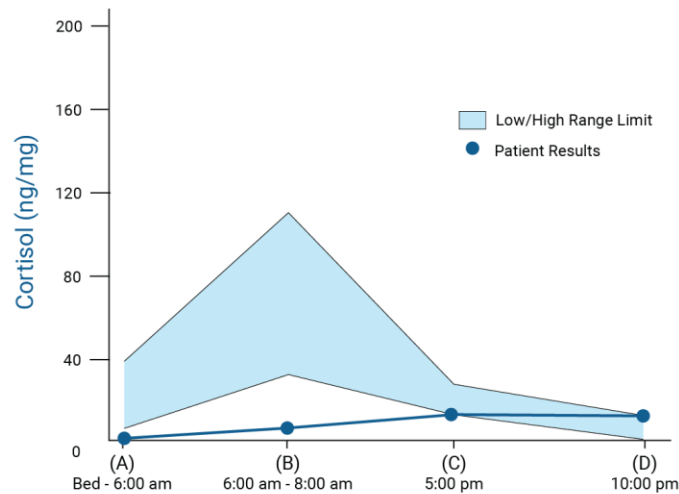
Low Cortisol?

Urine Free Cortisol Pattern



**Prednisone
Suppression**

Urine Free Cortisol Pattern

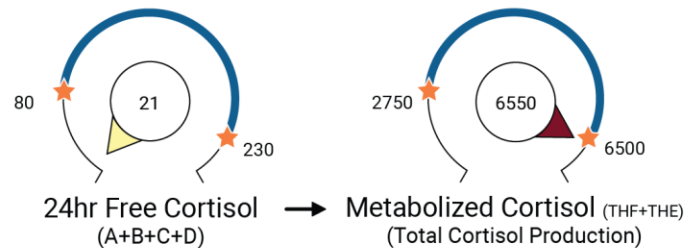
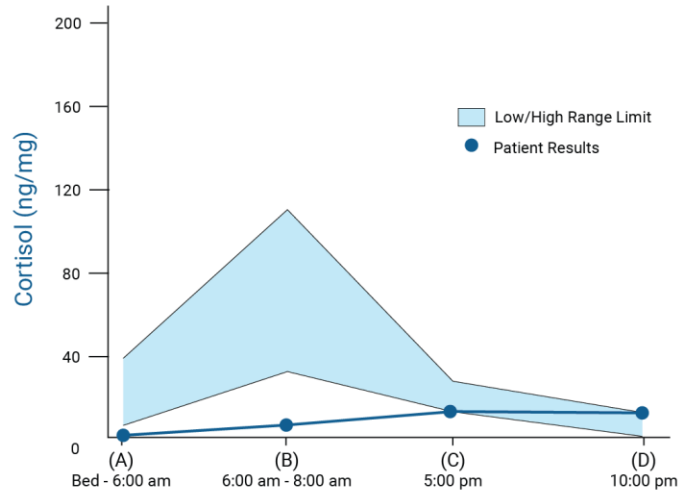


Thyroid Overdose

- fT3, T4 High
- Low TSH

Correcting Thyroid Overdose

Urine Free Cortisol Pattern

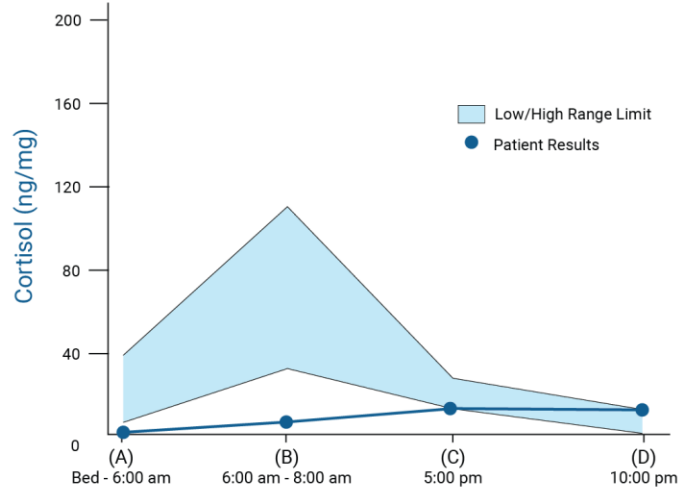


Thyroid Overdose

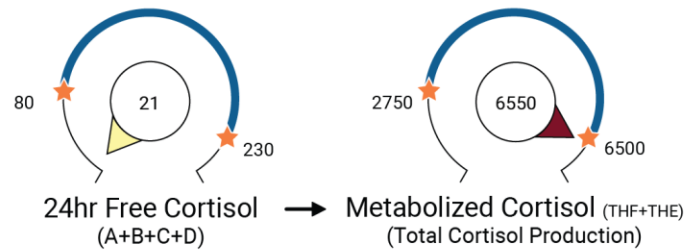
- fT3, T4 High
- Low TSH

Correcting Thyroid Overdose

Urine Free Cortisol Pattern



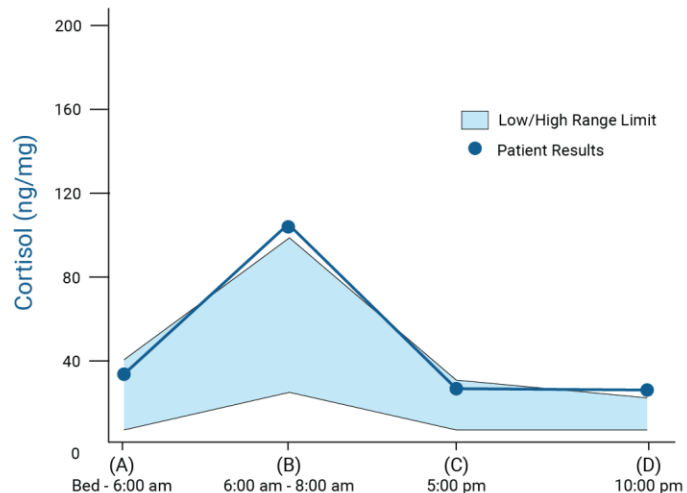
**Hyperthyroidism
increasing free
cortisol clearance**



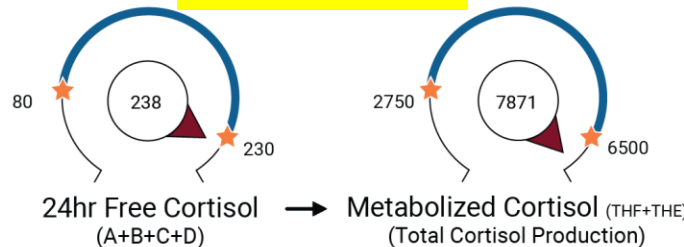
Thyroid Overdose

- **fT3, T4 High**
- **Low TSH**

Urine Free Cortisol Pattern



**Still have other work
to do for their health
and HPA Axis**



Proper Dose of T3/T4

- **Diurnal pattern restored**

Dried Urine

- 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)

DUTCH Complete

Serum

- Estrogen (production)
- Testosterone (production)
- DHEA (production)
- Progesterone (production)

DUTCH Plus

Saliva

- Cortisol (free pattern)
- Cortisol (CAR)

When does SERUM not provide adequate differentiating power?

1. Cortisol
2. Estradiol
3. Testosterone

If the methods are not carefully selected (LC-MS/MS)

You need LC-
MS/MS here for
serum

Data to support a simultaneous testosterone and estradiol assay in serum by LC-MS/MS

Ryan C. Schofield, Lakshmi V. Ramanathan, Melissa S. Pessin, Dean C. Carlow*

Department of Laboratory Medicine, Memorial Sloan Kettering Cancer Center, Center for Laboratory Medicine, 327 E 64th St., New York, NY, United States

1162

R.C. Schofield et al. / Data in Brief 20 (2018) 1160–1165

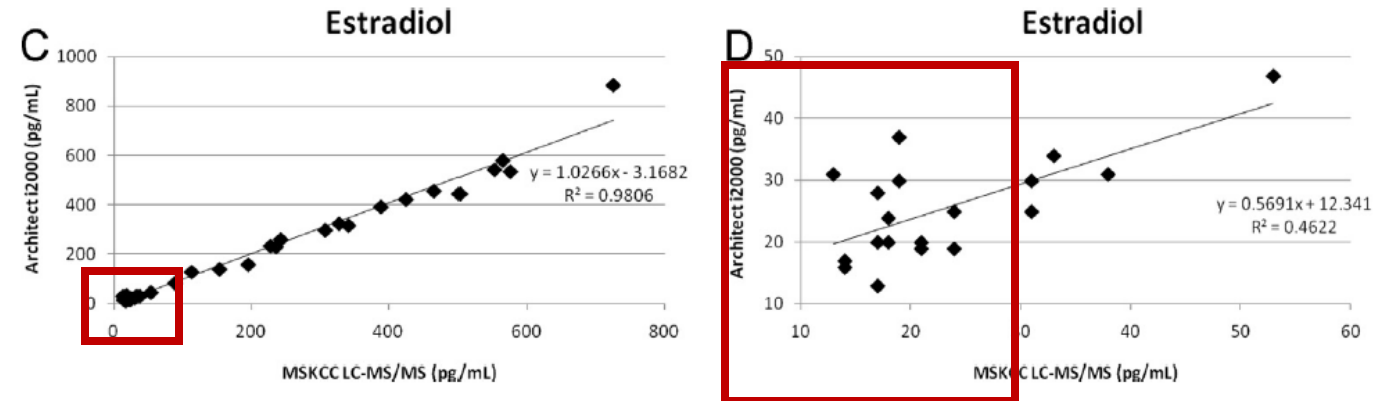


Fig. 2. (C) Correlation between the newly developed HPLC-MS/MS assay and the Architect i2000 immunoassay for estradiol; (D) Correlation between the newly developed HPLC-MS/MS assay and the Architect i2000 immunoassay at the low end of the analytical measurement range.

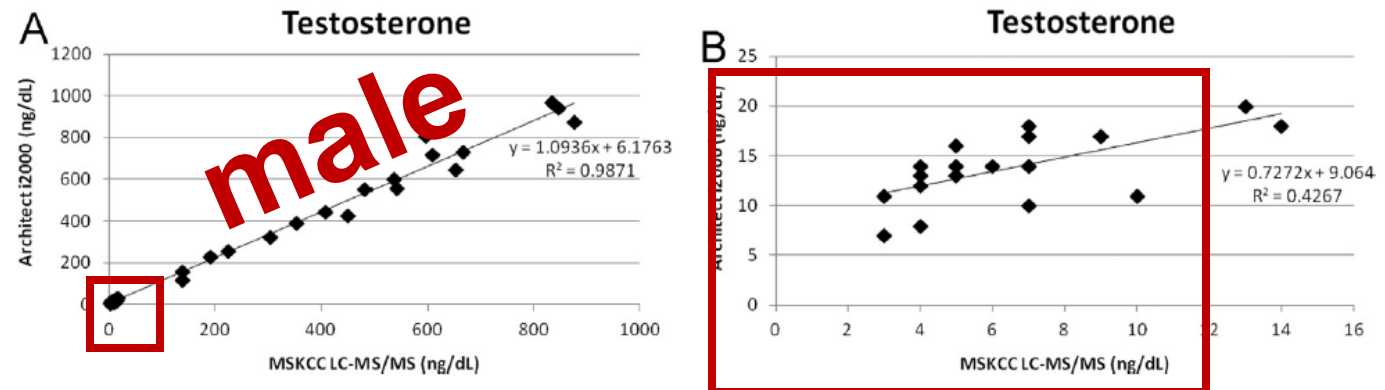


Fig. 1. (A) Correlation between the newly developed HPLC-MS/MS assay and the Architect i2000 immunoassay for testosterone; (B) Correlation between the newly developed HPLC-MS/MS assay and the Architect i2000 immunoassay at the low end of the analytical measurement range.

A lab's ranges tell you a lot about their test for E2

Lab Corp Serum E2 – standard assay

- Premenopausal Range 44-211pg/mL
- Postmenopausal Range 0-55pg/mL

“standard assay” = immunoassay, not LC-MS/MS

A lab's ranges tell you a lot about their test for E2

Lab Corp Serum E2 – standard assay

- Premenopausal Range 44-211pg/mL
- Postmenopausal Range 0-55pg/mL

Lab Corp Serum E2 – LC-MS/MS assay

- Premenopausal Range 70-300pg/mL
- Postmenopausal Range 0-15pg/mL

Premenopausal women make ~10X more E2 than PMP

Lab Corp Serum E2 – LC-MS/MS assay

- Premenopausal Range 70-300pg/mL
- Postmenopausal Range 0-15pg/mL

Premenopausal women make ~10X more E2 than PMP

**This distinction is lost with inadequately
accurate serum assays (not LC-MS/MS): ~4X**

Lab Corp Serum E2 – LC-MS/MS assay

- **Premenopausal Range 70-300pg/mL**
- **Postmenopausal Range 0-15pg/mL**

You need LC-
MS/MS here for
serum

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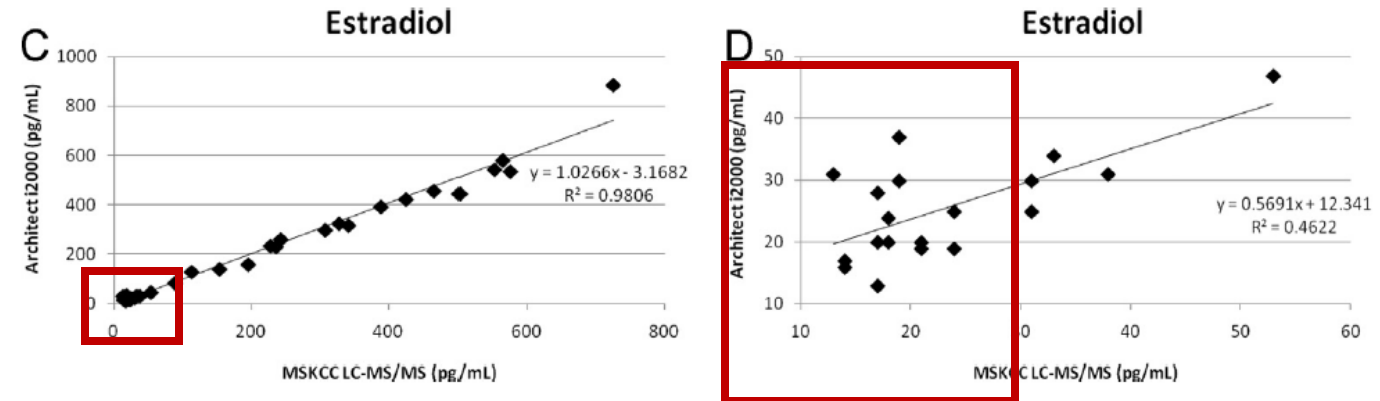


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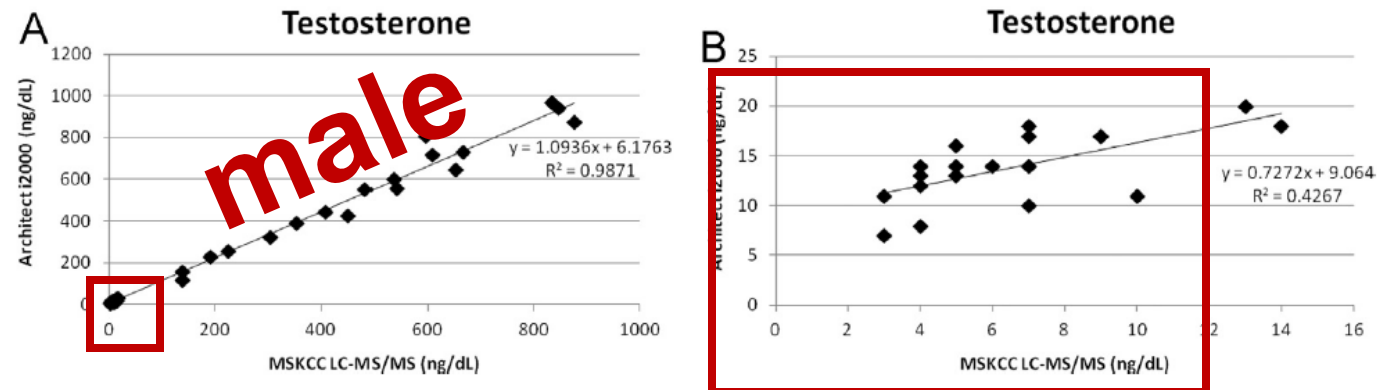


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You need LC-MS/MS here for serum

Data to support a simultaneous testosterone and estradiol assay in serum by LC-MS/MS

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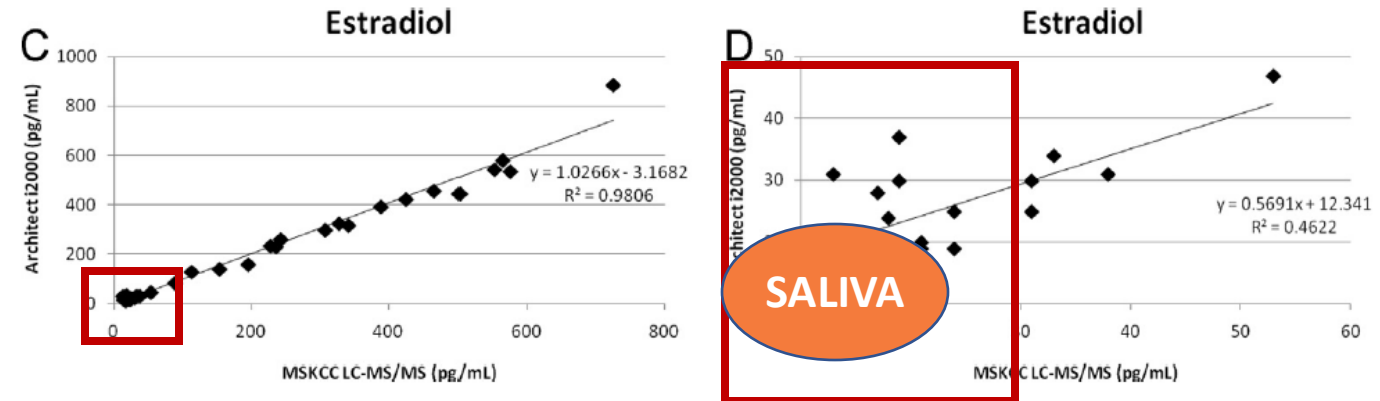


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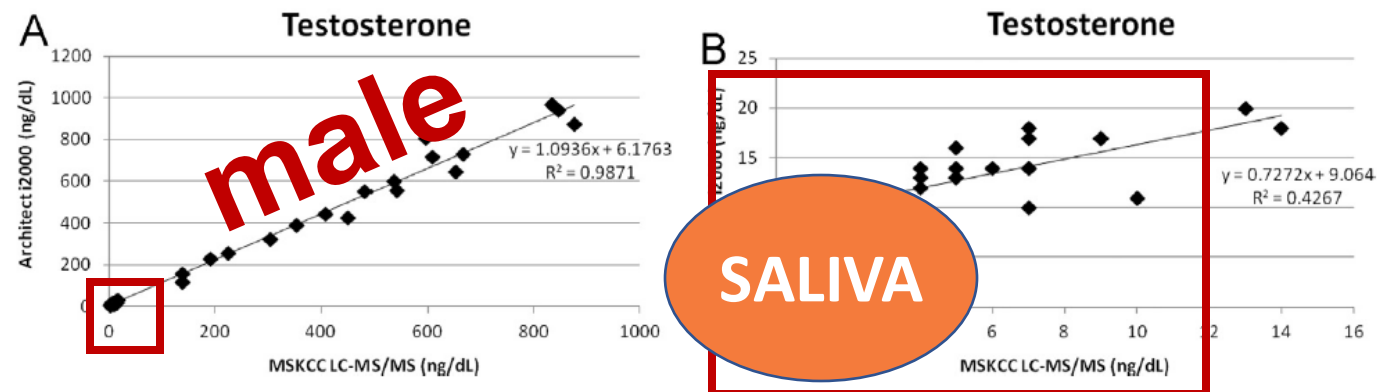
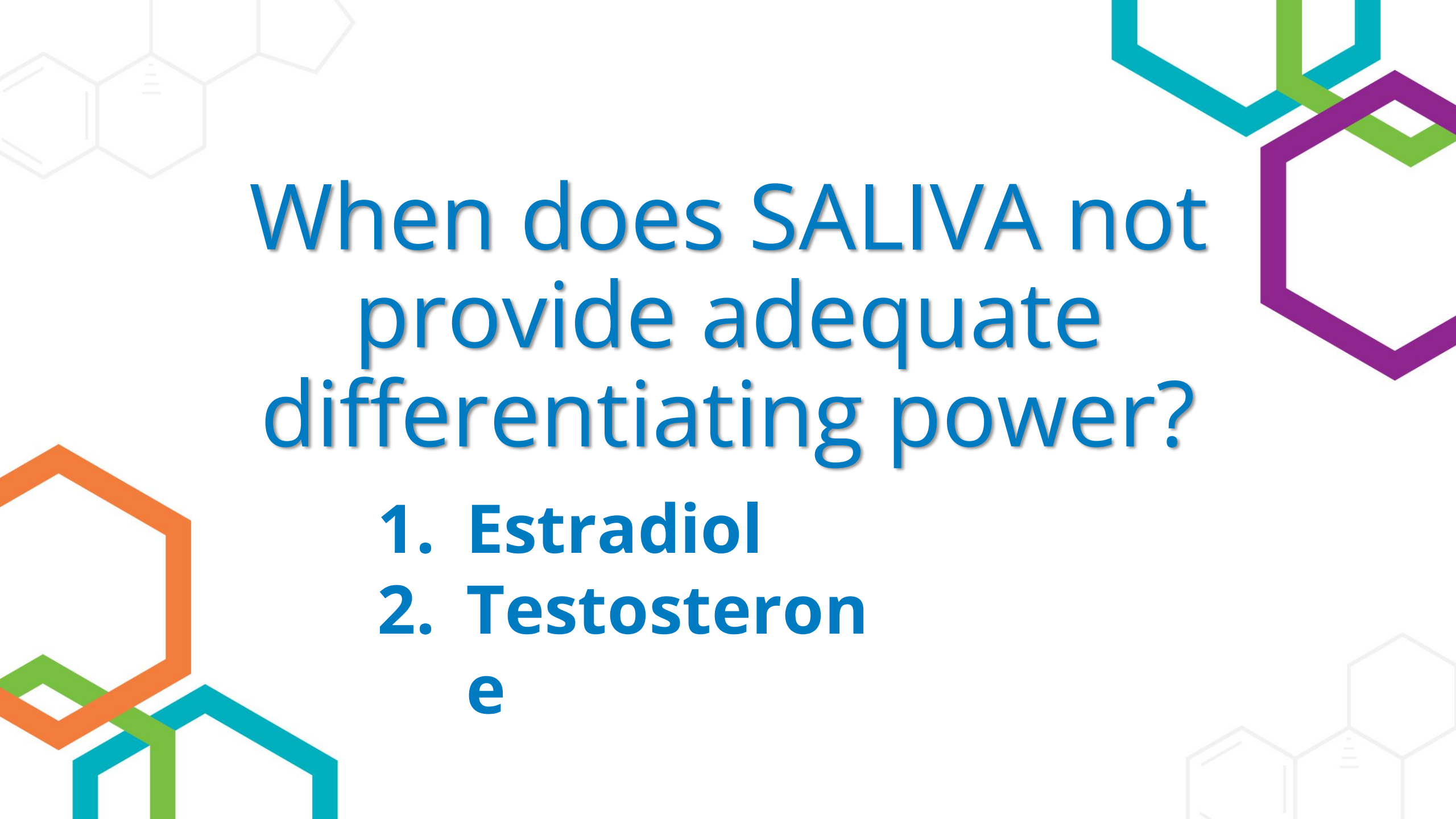


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When does SALIVA not
provide adequate
differentiating power?

1. Estradiol
2. Testosterone



**Why we have
avoided saliva for
measuring E2**

Reservations about low level salivary E2

Int J Adolesc Med Health 2018; 30(1): 20150126

Mazen Amatoury*, Jennifer W. Lee, Ann M. Maguire, Geoffrey R. Ambler
and Katharine S. Steinbeck

Utility of salivary enzyme immunoassays for measuring estradiol and testosterone in adolescents: a pilot study

This is the only
saliva-serum
correlation
published with a
commercially
available E2 assay.

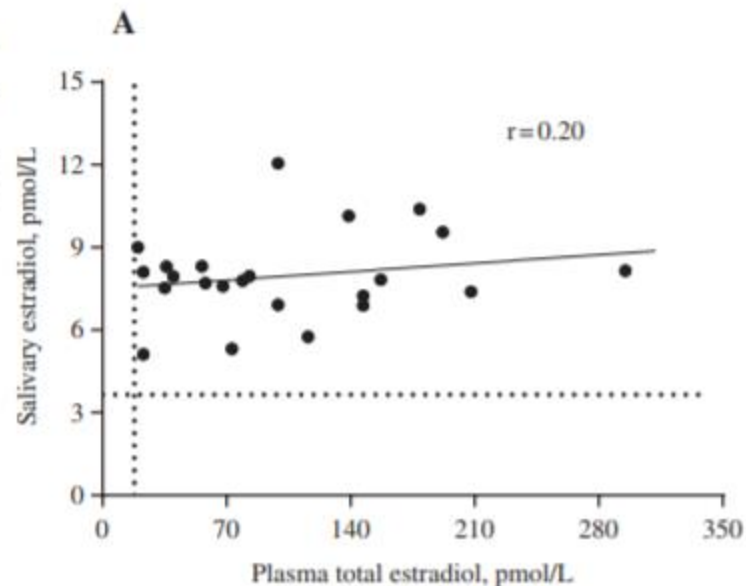
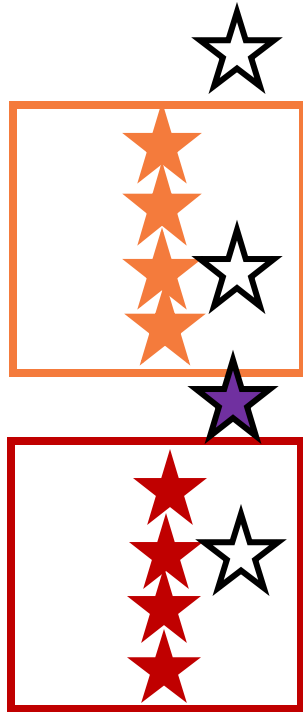


Figure 1: Linear regression showing associations between assayed saliva and plasma total estradiol (A) and testosterone (B). Dotted lines indicate assay lower limits of quantification (lowest calibrator concentration provided). * $p<0.001$.

★ Postmenopausal
★ Premenopausal

□ “Normal” Range
☆ Your patient

Estradiol



This conceptual lab test shows
“meaningful differentiation”

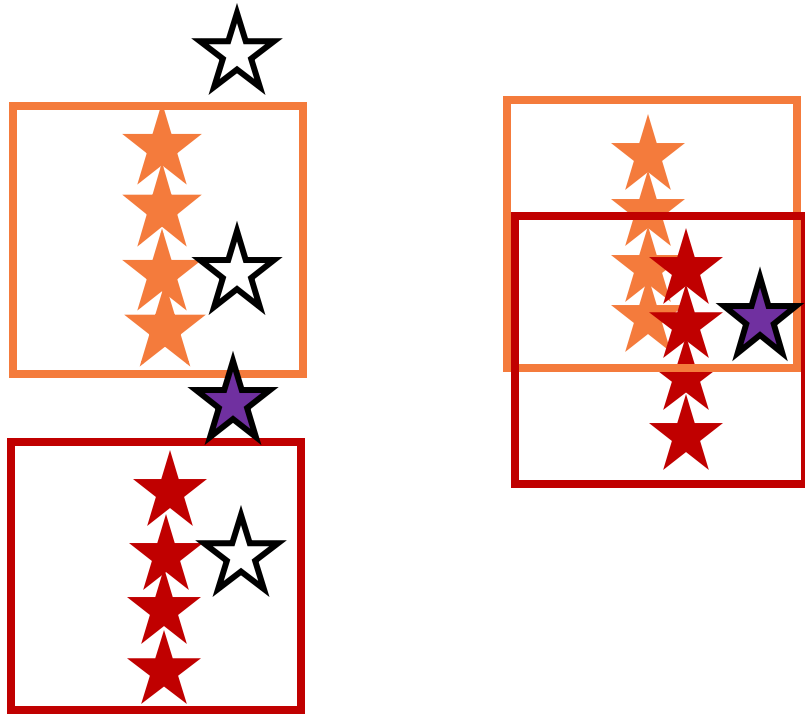
Test#1

Estradiol

★ Postmenopausal
★ Premenopausal



“Normal” Range
Your patient

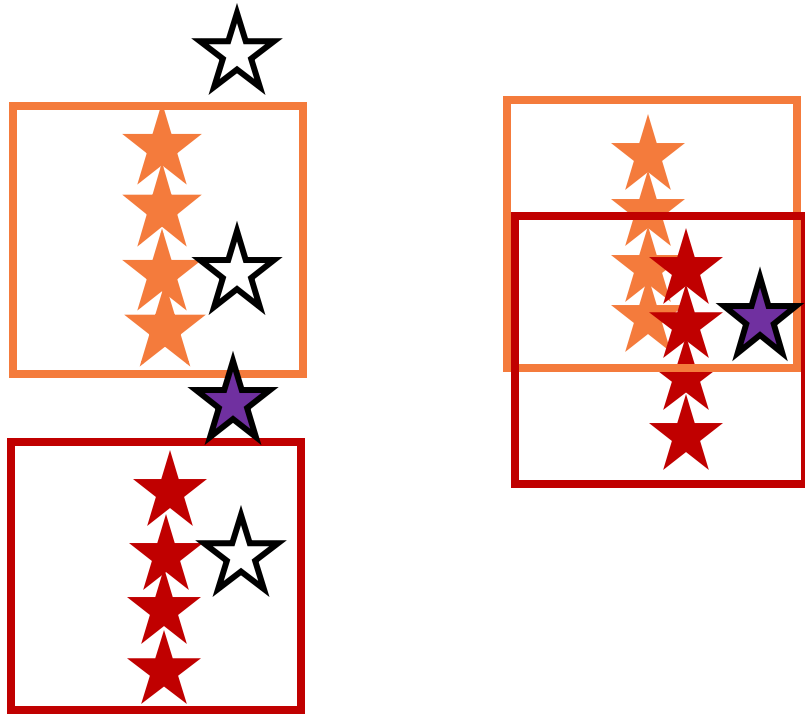


Test#1

Test#2

This lab test lacks
“meaningful differentiation”

Estradiol

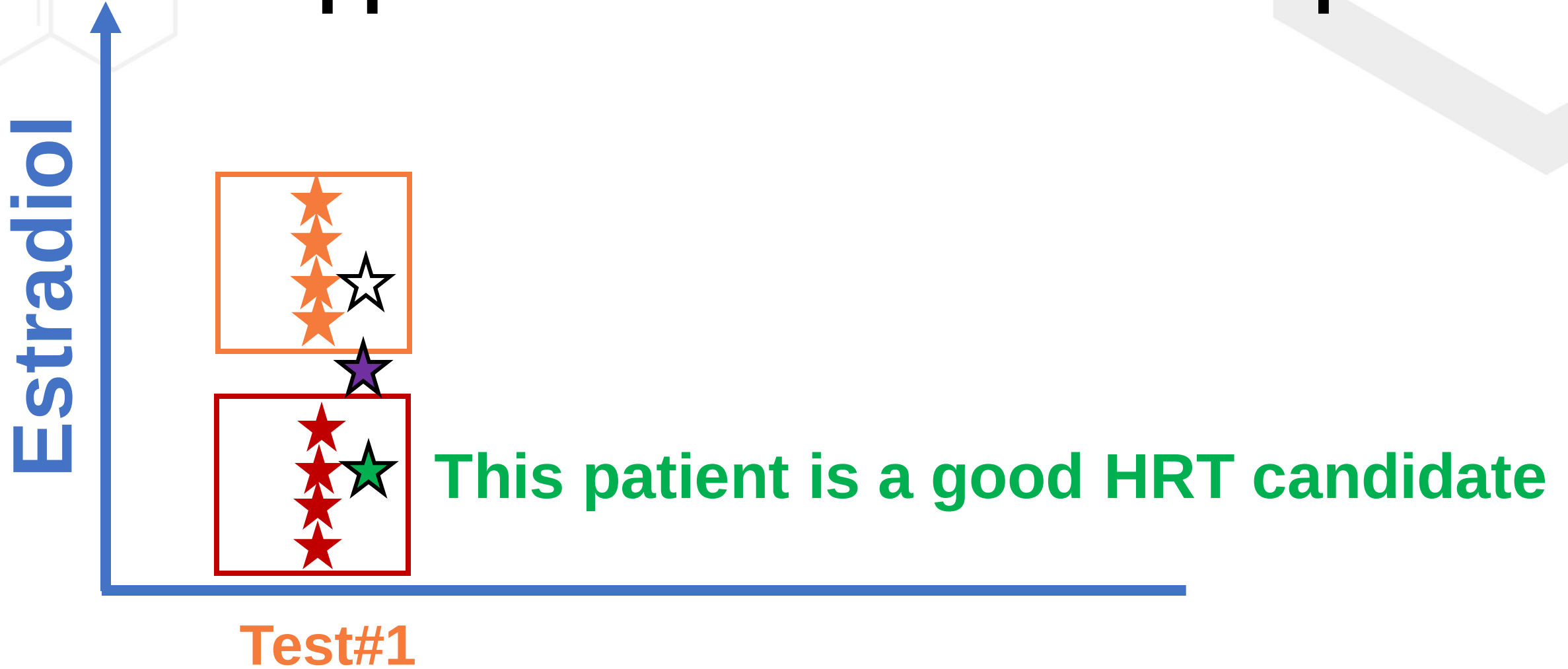


Test#1

Test#2

Is this patient a good
HRT candidate?

What happens when women take E2 patch?



What happens when women take E2 patch?

1. Climara FDA Prescribing Information. Drugs.com. (2019). Climara - FDA prescribing information, side effects and uses. Available at <https://www.drugs.com/pro/climara.html>.
2. Newman M, Stanczyk F, Zava D. Extraction Prior to Enzyme Immunoassay Gives Reliable Salivary Estradiol Monitoring during Estrogen Therapy.
Poster presented at: The Society for Gynecologic Investigation Annual Meeting; March 26-29, 2008; San Diego, CA. Note, 0.1mg “High Dose” Vivelle = 1.5, Climara = 1.7pg/mL
3. Newman M, Mayfield BP, Saltiel D, Stanczyk F. Monitoring Transdermal Estradiol Gel Therapy with a Validated Dried Urine Assay.
Poster presented at: The North American Menopause Society Annual Meeting; September 22-25, 2021; Washington, DC.

What happens when women take E2 patch?

	No Therapy	Low Dose	High Dose	
serum E2	<u>10</u>			pg/mL
saliva E2	<u>1.0</u>	?	?	pg/mL
urine E2	<u>0.35</u>			ng/mg

What happens when women take E2 patch?

		No Therapy	→	Low Dose	High Dose	
serum	E2	<u>10</u>	→	<u>25</u>		pg/mL
saliva	E2	<u>1.0</u>	→	<u>1.2</u>	?	pg/mL
urine	E2	<u>0.35</u>	→	<u>1.0</u>		ng/mg

What happens when women take E2 patch?

	No Therapy	Low Dose	High Dose	
serum E2	<u>10</u>	→ <u>25</u>	→ <u>100</u>	pg/mL
saliva E2	<u>1.0</u>	→ <u>1.2</u>	→ <u>1.5</u>	pg/mL
urine E2	<u>0.35</u>	→ <u>1.0</u>	→ <u>3.5</u>	ng/mg

What happens when women take E2 patch?

	No Therapy	High Dose
serum E2	<u>8-12</u>	<u>80-120</u> pg/mL
saliva E2	<u>0.8-1.2</u>	<u>1.2-1.8</u> pg/mL
urine E2	<u>0.3-0.5</u>	<u>2.8-4.2</u> ng/mg

What happens when women take E2 patch?

No
Therapy

High
Dose

saliva E2 0.8-1.2

1.2-1.8 pg/mL

What happens when women take E2 patch?

No
Therapy

High
Dose

serum E2 **8-12**

80-120 pg/mL

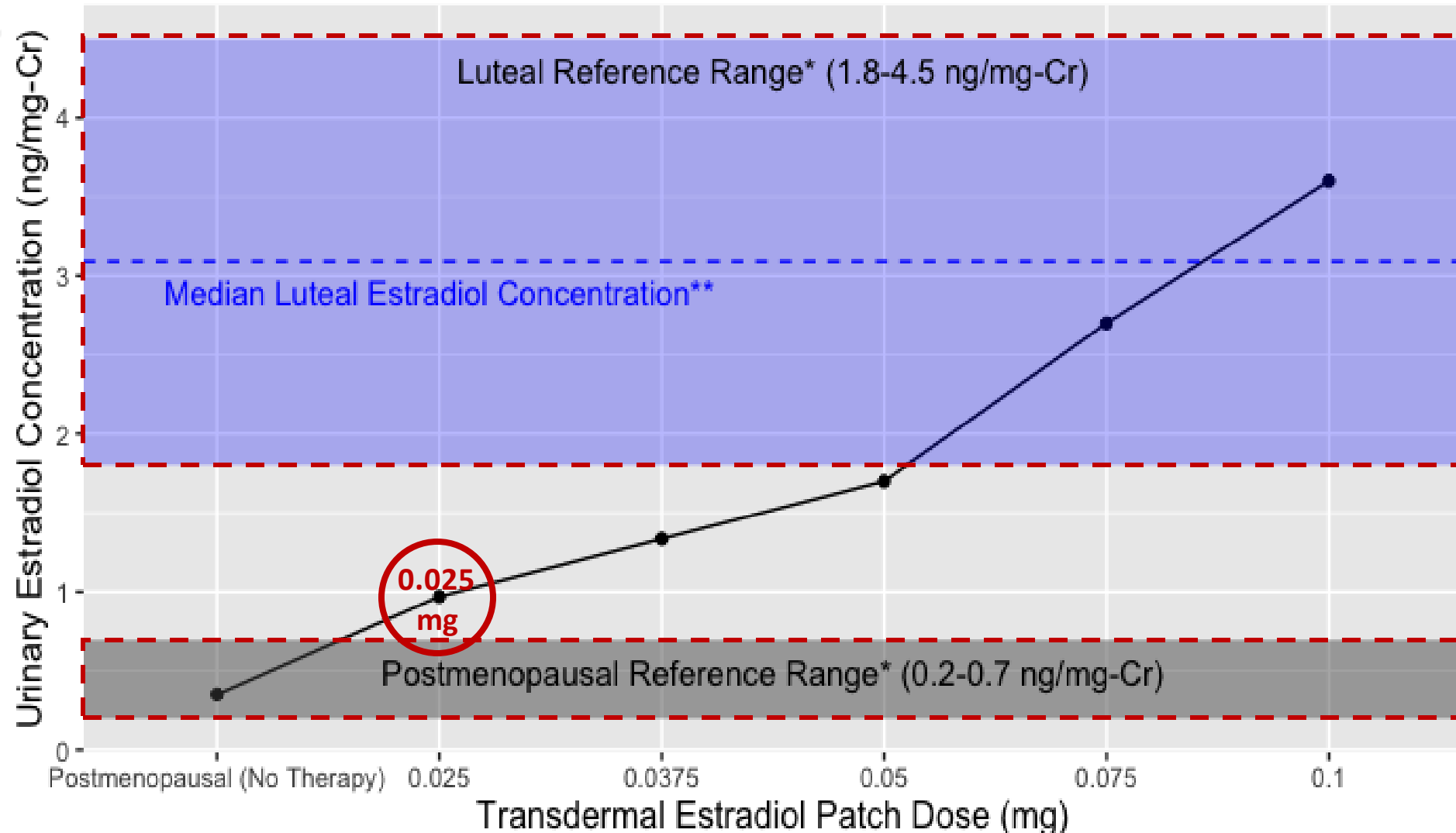
saliva E2 **0.8-1.2-1.8** pg/mL

urine E2 **0.3-0.5**

2.8-4.2 ng/mg

Patch Data in Dried Urine, NAMS 2021

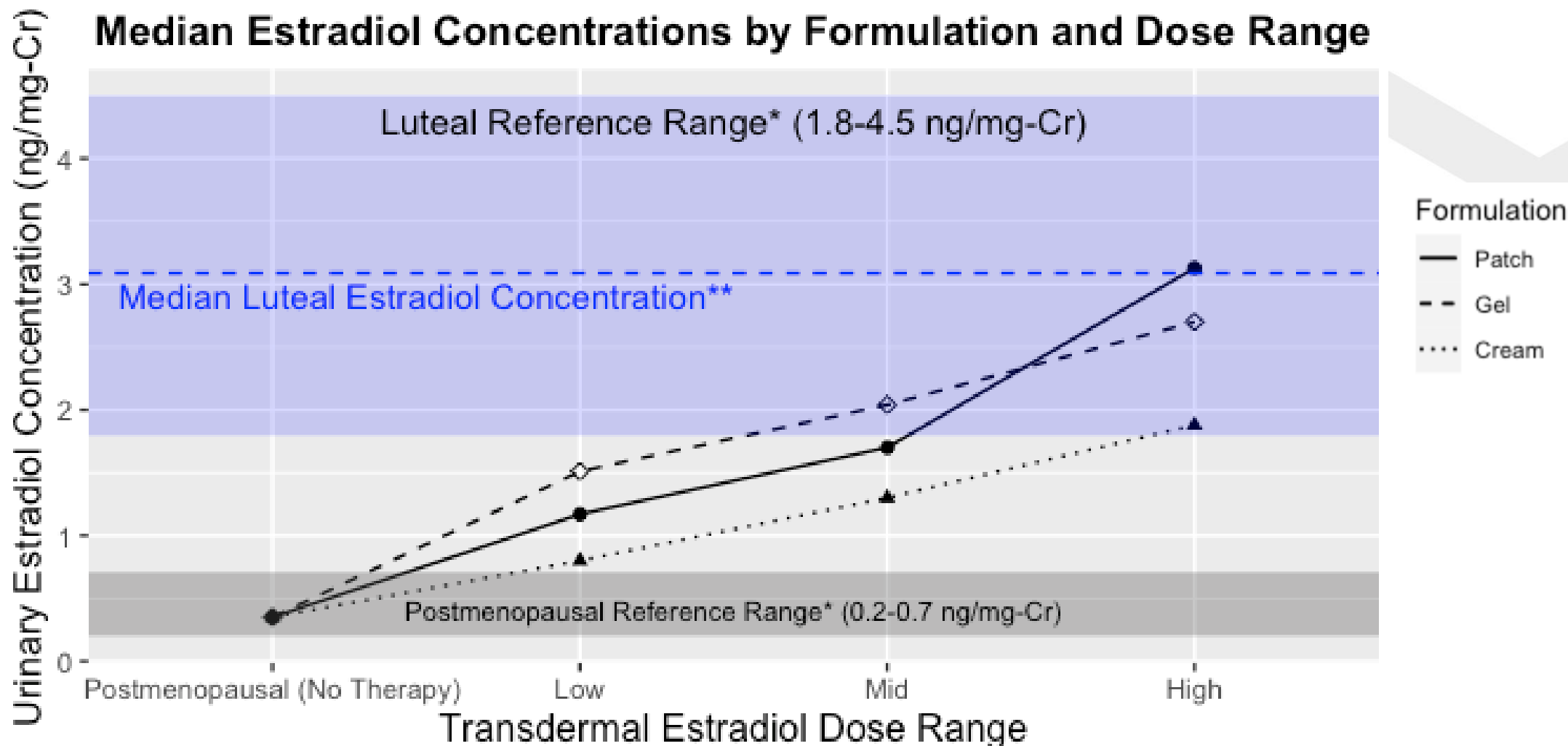
Median Estradiol Concentrations by Dose



*Reference ranges are those established by Precision Analytical, a CLIA-certified clinical laboratory.

**Median luteal estradiol concentration of premenopausal women included in the study

Accepted for publication (NAMS 2021)



The respective dose ranges (mg) for the estradiol cream, gel, and patch were

Low: 0.25-0.5 (cream), 0.25-0.5 (gel), 0.025-0.0375 (patch)

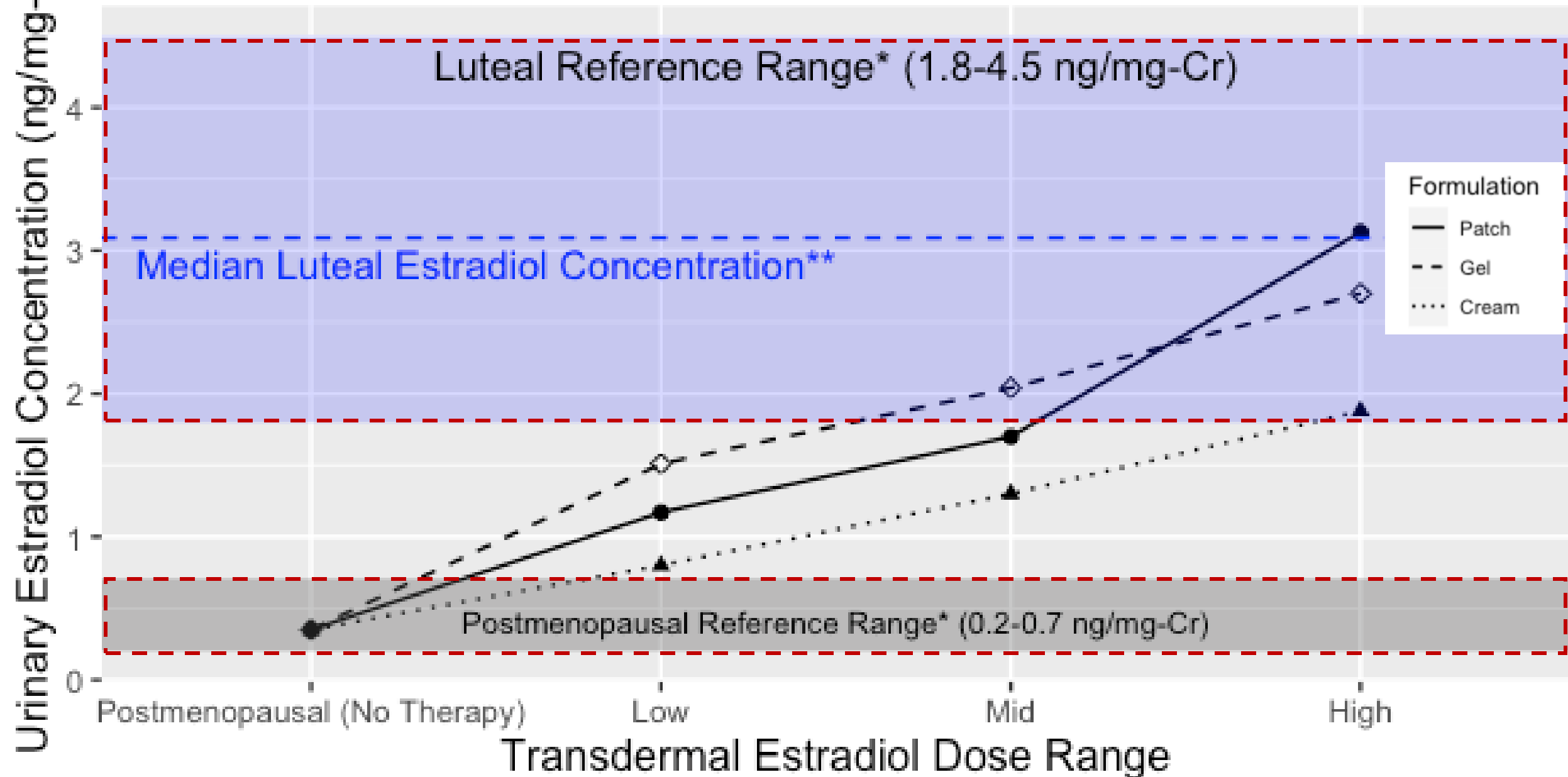
Mid: 0.75-1.25 (cream), 0.75-1.0 (gel), 0.05 (patch)

High: 1.5-3.0 (cream), 1.5-3.0 (gel), 0.075-0.1 (patch)

Low Dose Gel References

- 1 Archer DF, et al. Transdermal estradiol gel for the treatment of symptomatic postmenopausal women. *Menopause*. 2012; 19(6): 622-629.
- 2 Hedrick RE, et al. Transdermal estradiol gel 0.1% for the treatment of vasomotor symptoms in postmenopausal women. *Menopause*. 2009; 16(1): 132-140.
- 3 Divigel FDA Prescribing Information. Drugs.com. (2019). Divigel - FDA prescribing information, side effects and uses. [online] Available at: <https://www.drugs.com/pro/divigel.html>.
- 4 Simon JA, et al. Low dose of transdermal estradiol gel for treatment of symptomatic postmenopausal women. *Obstet Gynecol*. 2007 Mar;109(3): 588-96.
- 5 Elestrin FDA Prescribing Information. Drugs.com. (2019). Elestrin - FDA prescribing information, side effects and uses. [online] Available at: <https://www.drugs.com/pro/elestrin.html>.
- 6 EstroGel FDA Prescribing Information. Drugs.com. (2019). EstroGel - FDA prescribing information, side effects and uses. [online] Available at: <https://www.drugs.com/pro/estrogel.html>.
- 7 Archer DF, for the EstroGel Study Group. Percutaneous 17b-estradiol gel for the treatment of vasomotor symptoms in postmenopausal women. *Menopause*. 2003; 10(6): 516-521.
- 8 Yang TS, et al. A Clinical Trial of 3 Doses of Transdermal 17b-estradiol for Preventing Postmenopausal Bone Loss: A Preliminary Study. *J Chin Med Assoc*. 2007; 70(5): 200-206.
- 9 Brennan JJ, Lu Z, Whitman M, Stafiniak P, van der Hoop RG. Serum concentrations of 17beta-estradiol and estrone after multiple-dose administration of percutaneous estradiol gel in symptomatic menopausal women. *Ther Drug Monit*. 2001;23:134-8.
- 10 Newman M, Mayfield BP, Saltiel D, Stanczyk F. Monitoring Transdermal Estradiol Gel Therapy with a Validated Dried Urine Assay. Poster to presented at: The North American Menopause Society Annual Meeting; September 22-25, 2021; Washington, DC.

Patch, Gel, Cream Comparison: Urine



Dried Urine

- 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)

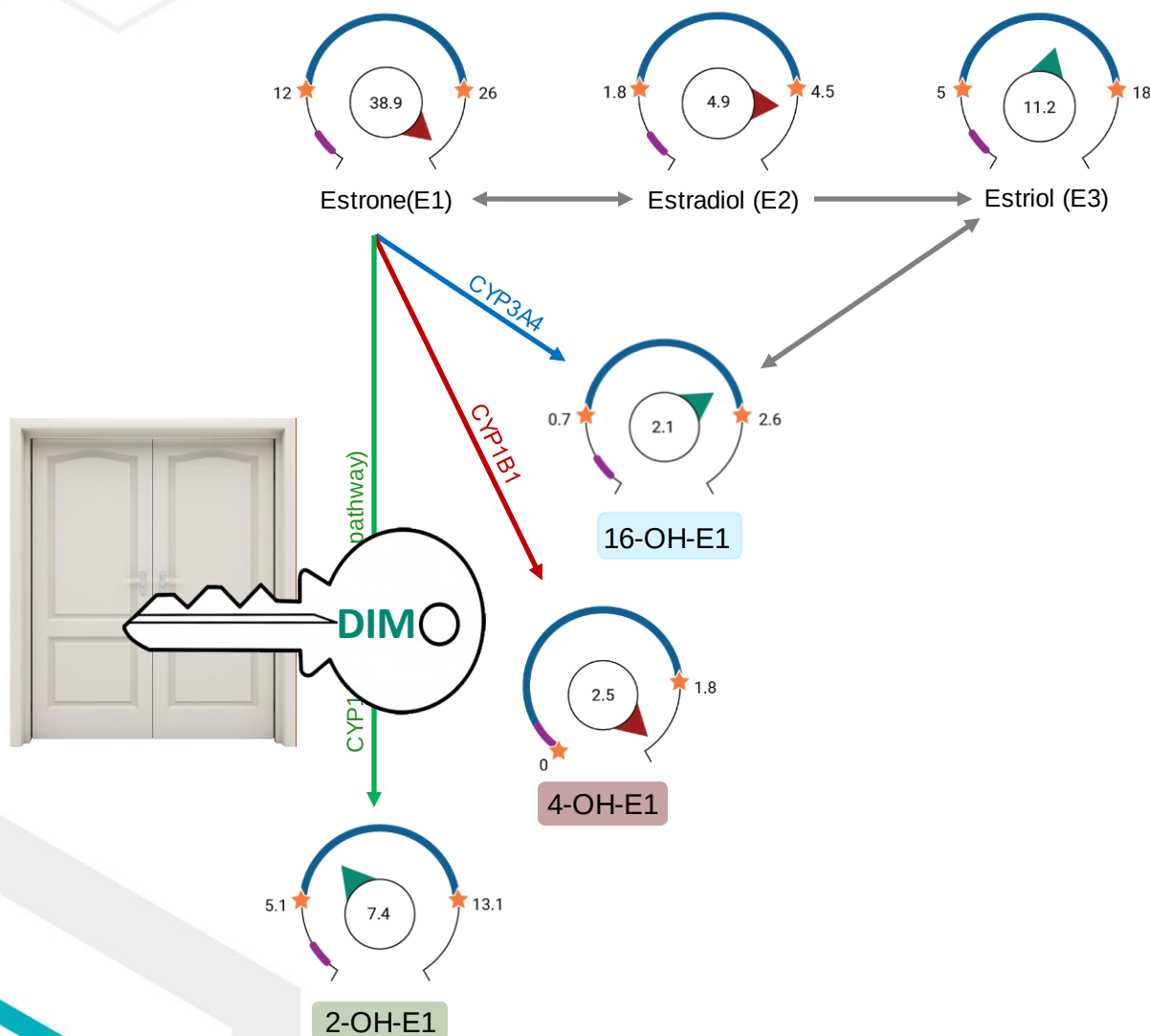
Serum

- Estrogen (production)
- Testosterone (production)
- DHEA (production)
- Progesterone (production)

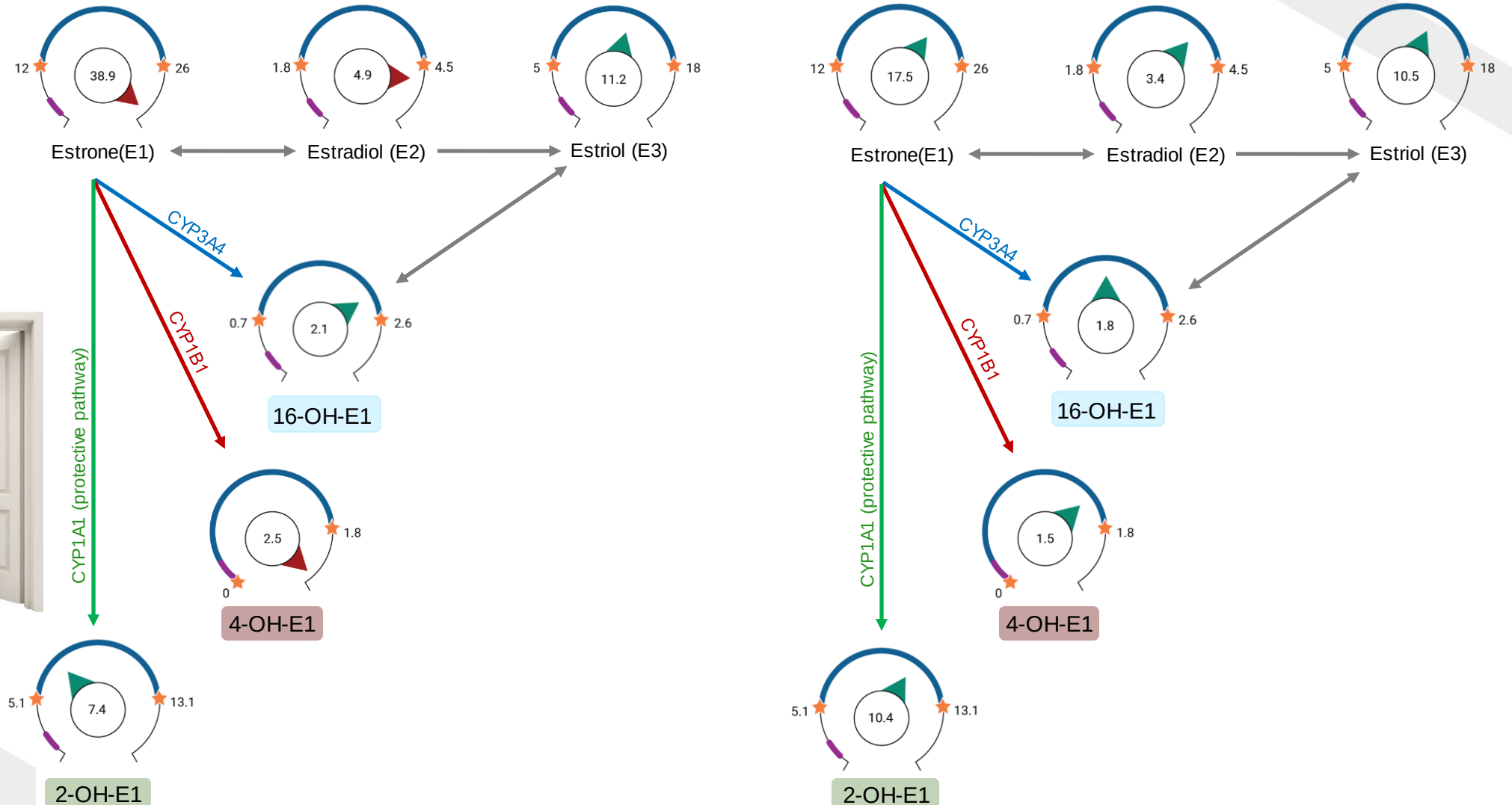
Saliva

- Cortisol (free)

Metabolites Provide a Fuller Picture



Before and After DIM



Estrogen Metabolites

Phase 1

- 2-OH-estrogens – thought to be protective
- 4-OH-estrogens – carcinogenic
- 16-OH-E1 – potent estrogen metabolite

Estrogen Metabolites

Phase 2

- **Methylation: 2-OH → 2-Methoxy via COMT**
- **Protective, especially for 4-OH → 4-methoxy**
 - **4-meth is tricky to measure compared to 2-meth**



Limited Meaningful Differentiation:

1. Serum cortisol
2. Salivary estrogen

Says the guy who owns a urine testing lab!





Limited Meaningful Differentiation:

1. Serum cortisol
2. Salivary estrogen
3. Urine testosterone

Dried Urine

- 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)

**Where is the
weak spot here?**

Dried Urine

- 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)

Where is the
weak spot here?

**Don't use spot urine hormone
tests with kidney or cortisol
clearance issues**

Dried Urine

- 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)

**Where is the
weak spot here?**



Urine Testing

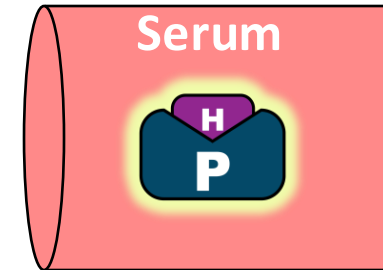


- **Proven accuracy for E2, Pg monitoring**
- **Testosterone not as strong in urine**

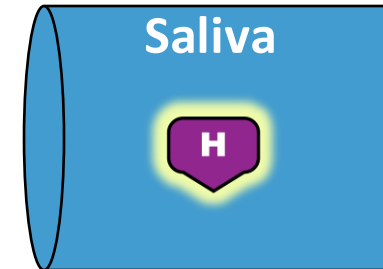
Urine Testing Assumes You Make



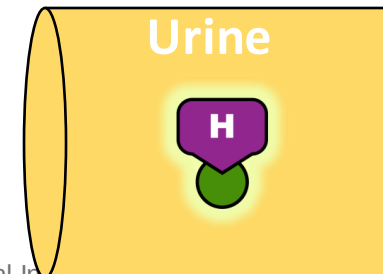
Protein-bound (SHBG, Albumin)



Free (unbound) Hormone



Hormone Conjugate (water-soluble)



Urine Testing Assumes You Make



Protein-bound (SHBG, Albumin)



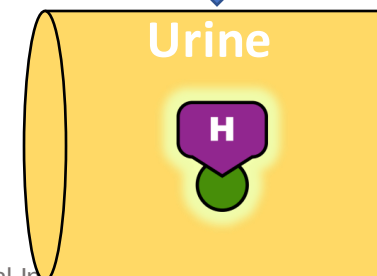
Protein-bound, free T
in equilibrium

Free (unbound) Hormone



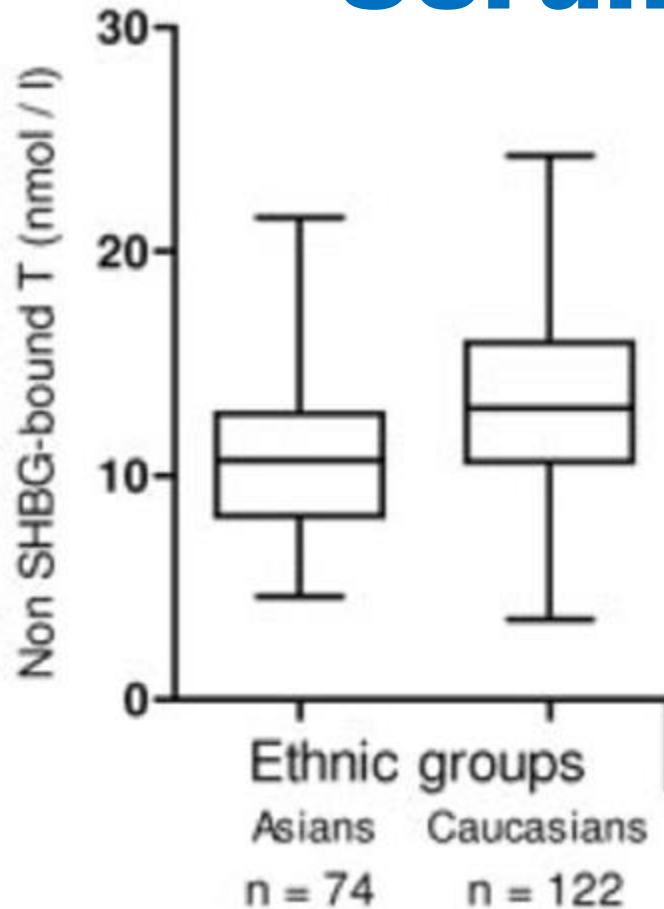
UGT Enzyme

Hormone Conjugate (water-soluble)

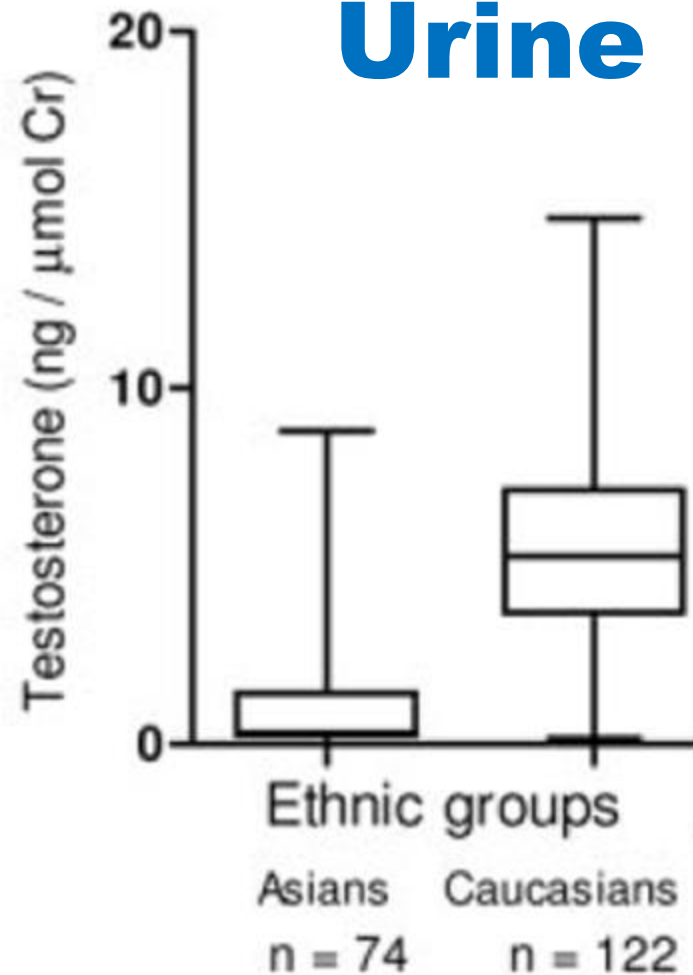


Urine artificially low in Asian patients

Serum



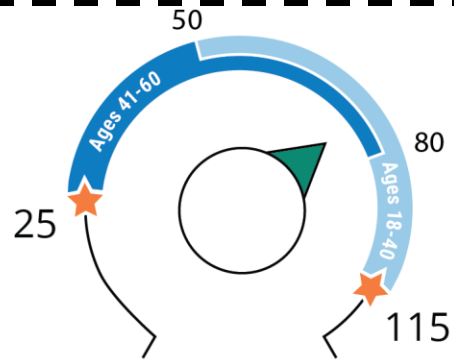
Urine



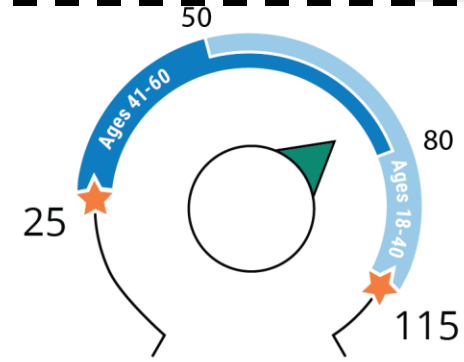
Differentiation?
Yes

Clinically meaningful?
No!

Patient A

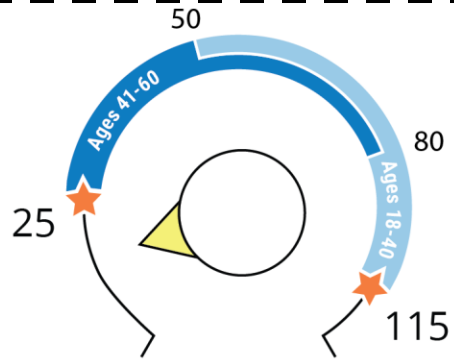


Testosterone

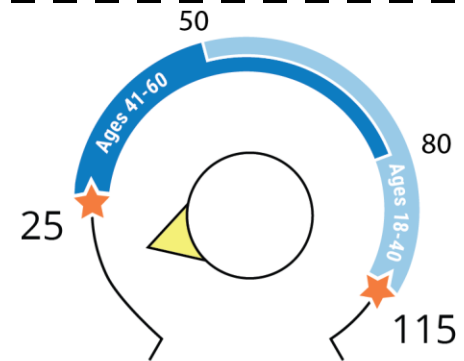


Epi-Testosterone

Patient B

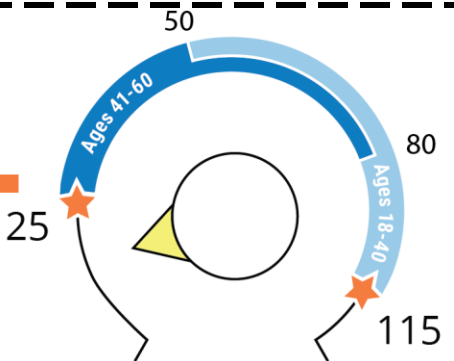


Testosterone

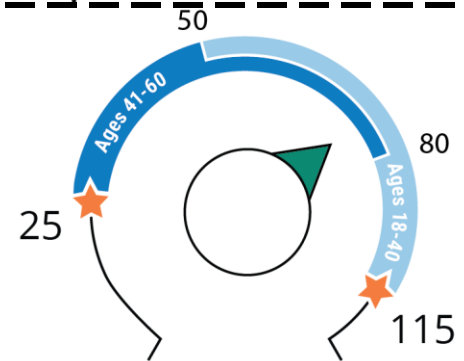


Epi-Testosterone

Patient UGT



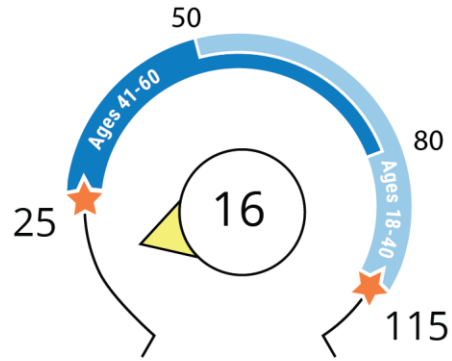
Testosterone



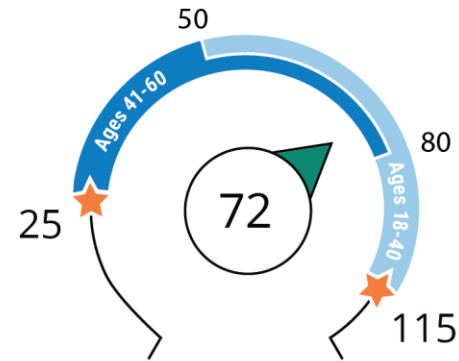
Epi-Testosterone

UGT Variant

What does it look like?



Testosterone



Epi-Testosterone

This is a complicated issue!

Urine Testing Caveat

- Serum T almost always best
- Never use urine T for Asian patients
- Other ethnicities can show this pattern, but the UGT variant is rare in non-Asians
- Metabolites in urine can be very helpful
 - DHT production (5 α -androstanediol)
 - Common patterns for PCOS patients

Dried Urine

- 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)

DUTCH Complete

Serum

- Estrogen (production)
- Testosterone (production)
- DHEA (production)
- Progesterone (production)

DUTCH Plus

Saliva

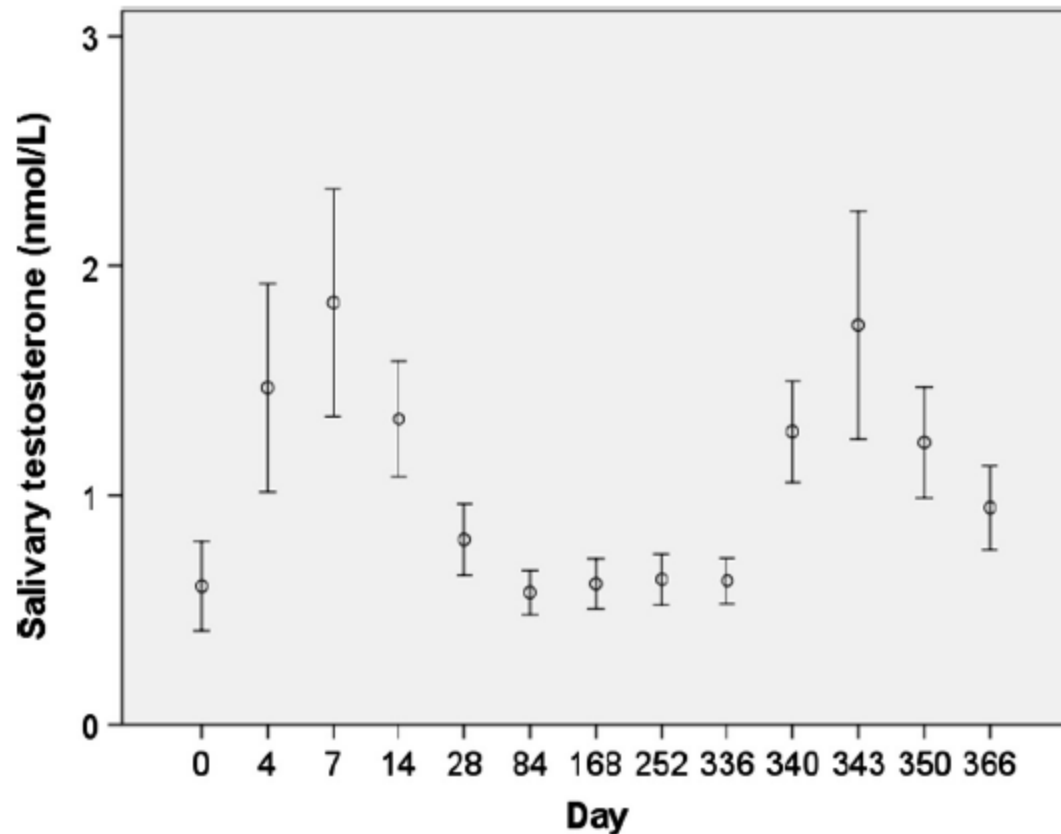
- Cortisol (free pattern)
- Cortisol (CAR)

The image features a white background with decorative hexagonal patterns in the corners. The top-left and bottom-right corners contain faint, light gray hexagonal outlines. The top-right and bottom-left corners contain thick, colorful hexagonal outlines in teal, green, and purple. The text is centered in a bold, blue, sans-serif font.

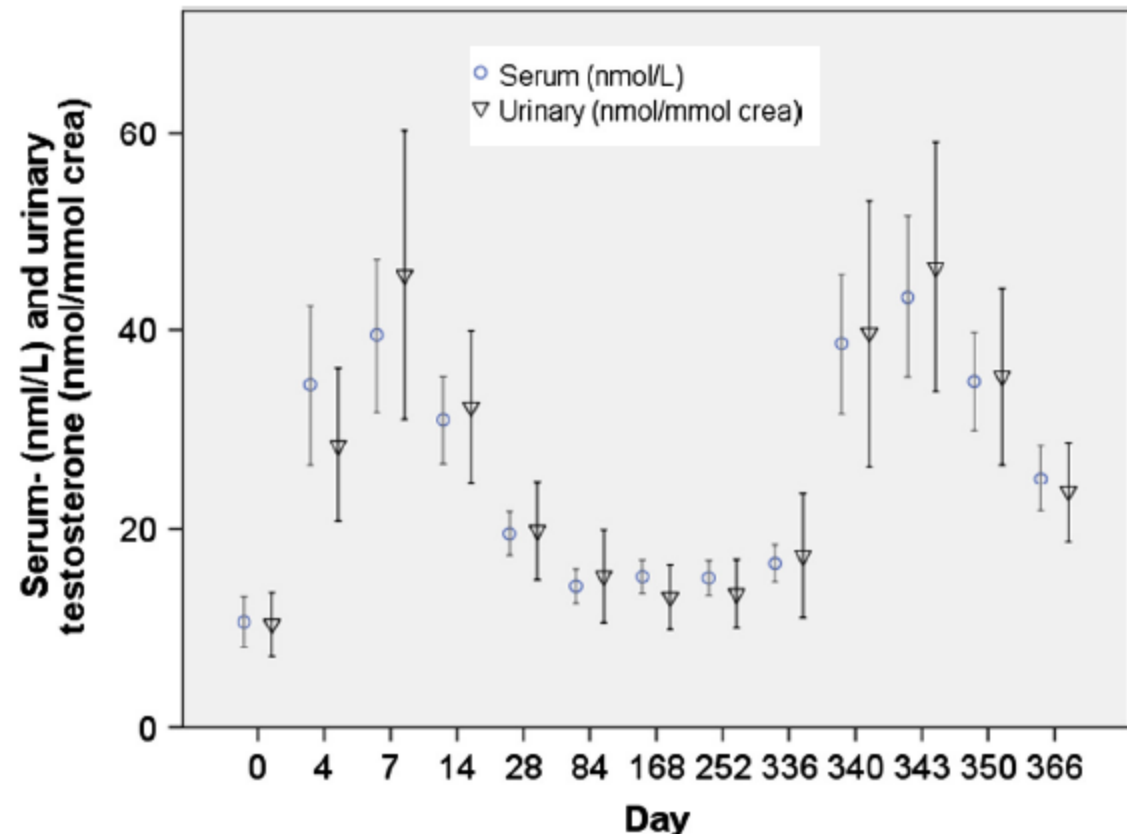
**What about
patients on HRT?**

Serum, Urine, & Saliva All Tell the Same Story RARELY with HRT

Figure 3 Testosterone concentrations (mean $\pm$ 95% CI) for all measured values in saliva (left) and serum and urine (right) in patients during testosterone undecanoate injections every 12th week during 12 months and first and last injections.



Error Bars: 95% CI

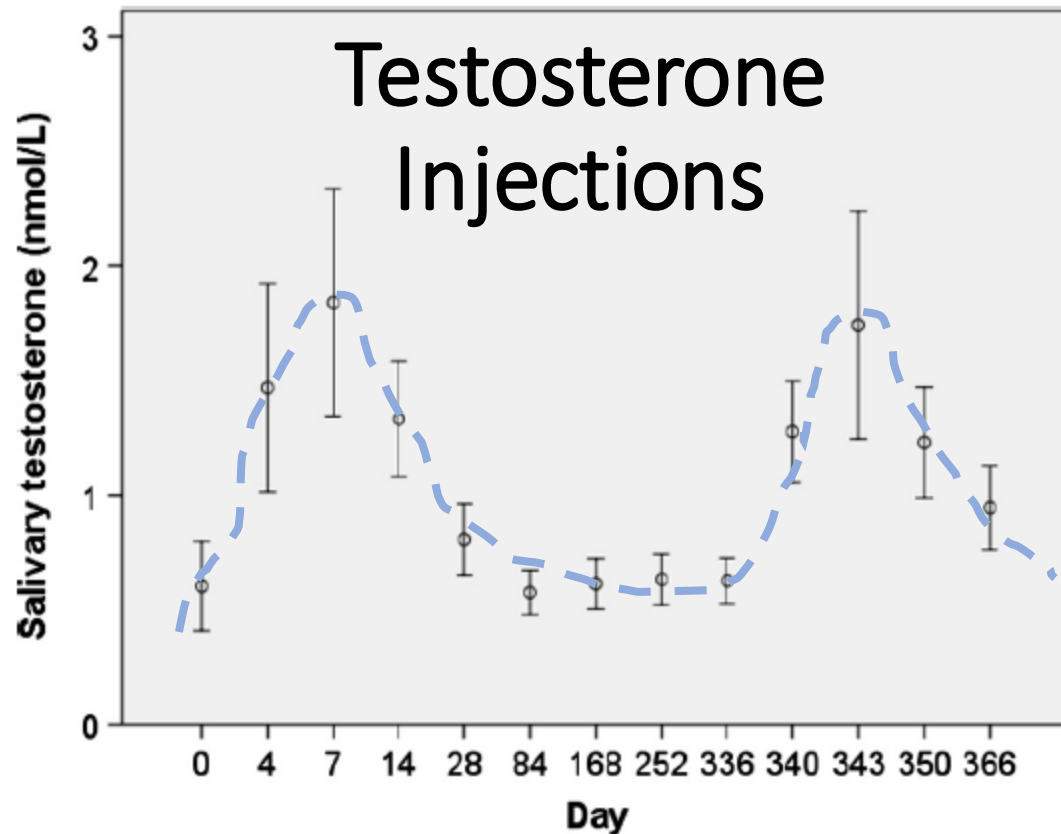


Error Bars: 95% CI

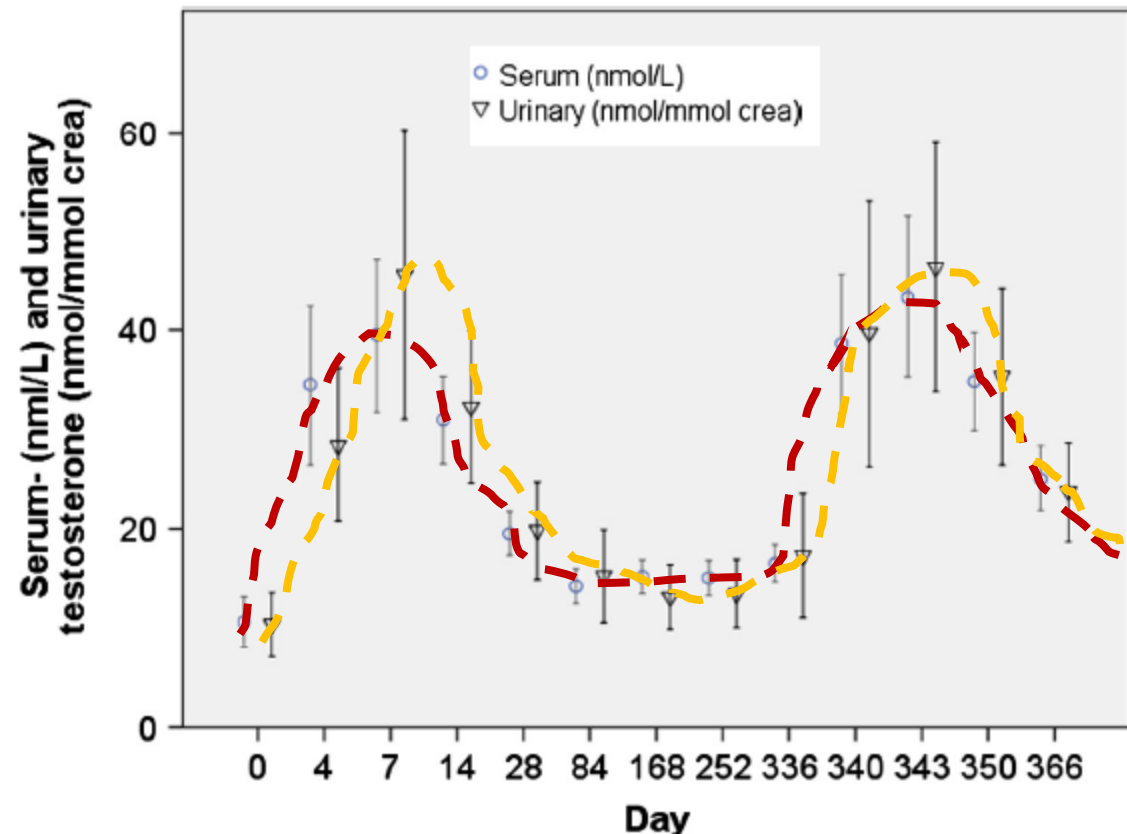
Andrology, 2018, 6, 86–93

Serum, Urine, & Saliva All Tell the Same Story RARELY with HRT

Figure 3 Testosterone concentrations (mean $\pm$ 95% CI) for all measured values in saliva (left) and serum and urine (right) in patients during testosterone undecanoate injections every 12th week during 12 months and first and last injections.



Error Bars: 95% CI



Error Bars: 95% CI

Andrology, 2018, 6, 86–93



The BHRT Monitoring Matrix!

**The search for meaningful differentiation
when using HRT**

MONITORING (B)HRT WITH LAB TESTING

Can Serum or Dried Urine, as a standalone test, effectively monitor HRT?

✓ Yes

✗ No

? Maybe

Oral Progesterone (OMP)	Estradiol (E2) Patches	E2 Gels & Creams (Skin)	Vaginal E2 & Testosterone (T)	Vaginal Progesterone (Pg)	Transdermal (TD) Testosterone	Testosterone Injections & Pellets
✓ DRIED URINE	✓ DRIED URINE	✓ DRIED URINE	✓ DRIED URINE	✗ DRIED URINE	? DRIED URINE	? DRIED URINE
Dried urine provides useful feedback when using OMP in women with PMP sleep disturbances. 5a (more active) and 5b metabolites are measured to individualize OMP dosing. OMP's sleep effects are via its 5a metabolites, predominately allopregnanolone binding to the GABA receptor. No lab test reflects OMP's effect on the endometrium.	Values between the top of the postmenopausal range and the lower limit of the premenopausal 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 daily up and down E2 patterns. This is particularly helpful with gels and creams that may have serum values that change rapidly over time.	4-spot dried urine methods may work uniquely well, if a special method is used to remove potential contamination for E2 and T. Very low doses may impact local tissue without increasing lab values. For local (not systemic) E2 therapy, keep urine E2 in PMP range.	Vaginal E2, Pg, and T are systemically absorbed. If placed in the top 1/3 of the vagina, a higher dose will get to the uterus (uterine 1st pass effect), which may be helpful for Pg, but not E2.	Pg is measured indirectly in urine by measuring pregnanediols. These metabolites may be underrepresented when Pg is taken vaginally. Serum Pg seems to increase to a higher degree than urine metabolites with vaginal Pg application.	Levels generally parallel changes in serum and clinical outcomes (increased lean body mass, erythrocytosis, etc. in men). Epi-testosterone (Epi-T) values can be used to assess gonadal suppression due to TRT (Epi-T levels in men decrease as TRT increases and are <10ng/mg with complete suppression). Urine testosterone does not correlate as reliably to T serum values, compared to E2 and other tests. Urine testing is best suited as a complimentary test to serum for T and should not be used solely for TRT decisions.	Injections and pellets increase levels, as expected, but the increase may exceed what is seen in serum testing. Dried urine allows for monitoring both the dosing of hormones as well as metabolic patterns.
✗ SERUM	✓ SERUM	? SERUM	✓ SERUM	? SERUM	✓ SERUM	✓ SERUM
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.	Serum testing is well suited for use with these types of therapies. Results increase with increased dosing in a fairly linear fashion. Most recommendations are to push serum E2 levels to 20-40pg/mL for clinical impact.	The only published data for E2 creams shows serum results move up and down within a few hours, so serum testing can easily underestimate clinical impact. Dried urine results average out the daily up and down pattern and may be a better option.	Serum results rise quite dramatically with what may seem like modest doses due to the high uptake of hormones across the mucosal membrane. However, values may rise and fall quickly, so be careful with the interpretation of both low and high results.	Serum values increase with dosing and likely represent systemic exposure to Pg. However, the uterine first-pass effect saturates the uterus with high levels of Pg (which may be desirable) and serum does not reflect uterine hormone levels.	A great deal of published research shows that serum levels reflect clinical changes in both men and women taking TD T. Be aware of potential up and down patterns throughout the day, but serum is the best tool for monitoring doses of TD T in both men and women.	Serum testing is well suited for use with these types of therapies. Results increase with increased dosing in a fairly linear fashion. Test injections halfway between doses or right before a dose.
✗ SALIVA	The literature does not support salivary testing's use for monitoring TD hormone creams. The saliva data is limited and, in fact, there are no saliva testing outcome studies using TD creams, injections, estradiol patches, oral estradiol, or vaginal hormones. While salivary testing is the gold standard for free cortisol measurement, avoiding its use for monitoring HRT is advised. For situations where saliva testing may parallel the clinical impact, dried urine or serum testing are better options (see above).					
✗ Oral Estradiol, Estradiol Pellets, or Sublingual Hormones	Though not recommended, if you choose to use either oral estradiol or estradiol pellets, serum testing can monitor both, whereas dried urine should only be used with pellet therapy. Sublingual hormones may be used in some situations but lab monitoring is not helpful in optimizing doses.					
✗ Transdermal Progesterone	In PMP women, the evidence does not support TD Pg's use to protect the endometrium. When prescribed, laboratory monitoring is not helpful for TD Pg dosing.					

MONITORING (B)HRT WITH LAB TESTING

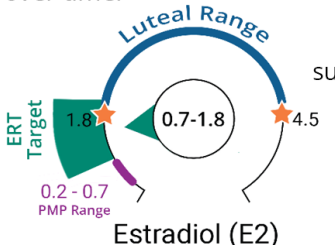
Tutorials available at www.dutchtest.com/videos/hormone-tutorials

Can serum or DUTCH, as a standalone test, effectively monitor HRT?

✓ Yes

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Oral Progesterone (OMP)	Estradiol (E2) Patches	E2 Gels & Creams (Skin)	Vaginal E2 & Testosterone (T)	Vaginal Progesterone (Pg)	Transdermal (TD) Testosterone	Testosterone Injections & Pellets
✓ DUTCH	✓ DUTCH	✓ DUTCH	✓ DUTCH	✗ DUTCH	? DUTCH	? DUTCH
The DUTCH Test® provides useful feedback when using OMP in women with PMP sleep disturbances. 5a (more active) and 5b metabolites are measured to individualize OMP dosing. OMP's sleep effects are via its 5a metabolites, predominately allopregnanolone binding to the GABA receptor. No lab test reflects OMP's effect on the endometrium.	Values between the top of the postmenopausal range and the lower limit of the premenopausal 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. DUTCH is preferred over serum because in addition to metabolites, dried urine averages out the daily up and down E2 patterns. This is particularly helpful with gels and creams that may have serum values that change rapidly over time.  The aggregate clinical data suggests that a serum (LC-MS/MS) E2 level of ~20-40pg/mL improves clinical outcomes (VMS, VVA, BMD). This approximates a DUTCH value of ~0.7-1.8ng/mg.		The DUTCH Test® is unique in that it removes potential contamination, and monitoring is helpful with E2 and T. Very low doses may impact local tissue without increasing lab values. For local (not systemic) E2 therapy, keep urine E2 in PMP range.	Pg is measured indirectly in urine by measuring pregnanediols. These metabolites may be underrepresented when Pg is taken vaginally. Serum Pg seems to increase to a higher degree than urine metabolites with vaginal Pg application.	Levels generally parallel changes in serum and clinical outcomes (increased lean body mass, erythrocytosis, etc. in men). Epi-testosterone (Epi-T) values can be used to assess gonadal suppression due to TRT (Epi-T levels in men decrease as TRT increases and are <10ng/mg with complete suppression).	Injections and pellets increase levels, as expected, but the increase may exceed what is seen in serum testing. DUTCH allows for monitoring both the dosing of hormones as well as metabolic patterns.
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How to Navigate the HRT Monitoring Maze

- What is the hormone? Pg? E2? T?
- What is the Route of Administration (ROA)?
- What lab tests (if any) provide meaningful differentiation in evaluating potential therapy changes?



HRT Monitoring Truths

- Some HRT scenarios do NOT have lab options that provide meaningful differentiation
- Serum, Urine, and Saliva all have serious limitations
- Pg, E2, and T should be examined independently for best practices. **Almost nothing is true of all three hormones as it relates to HRT and testing.**

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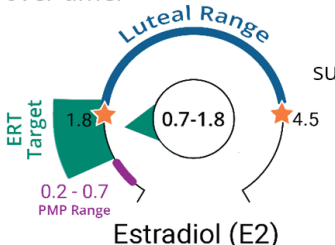
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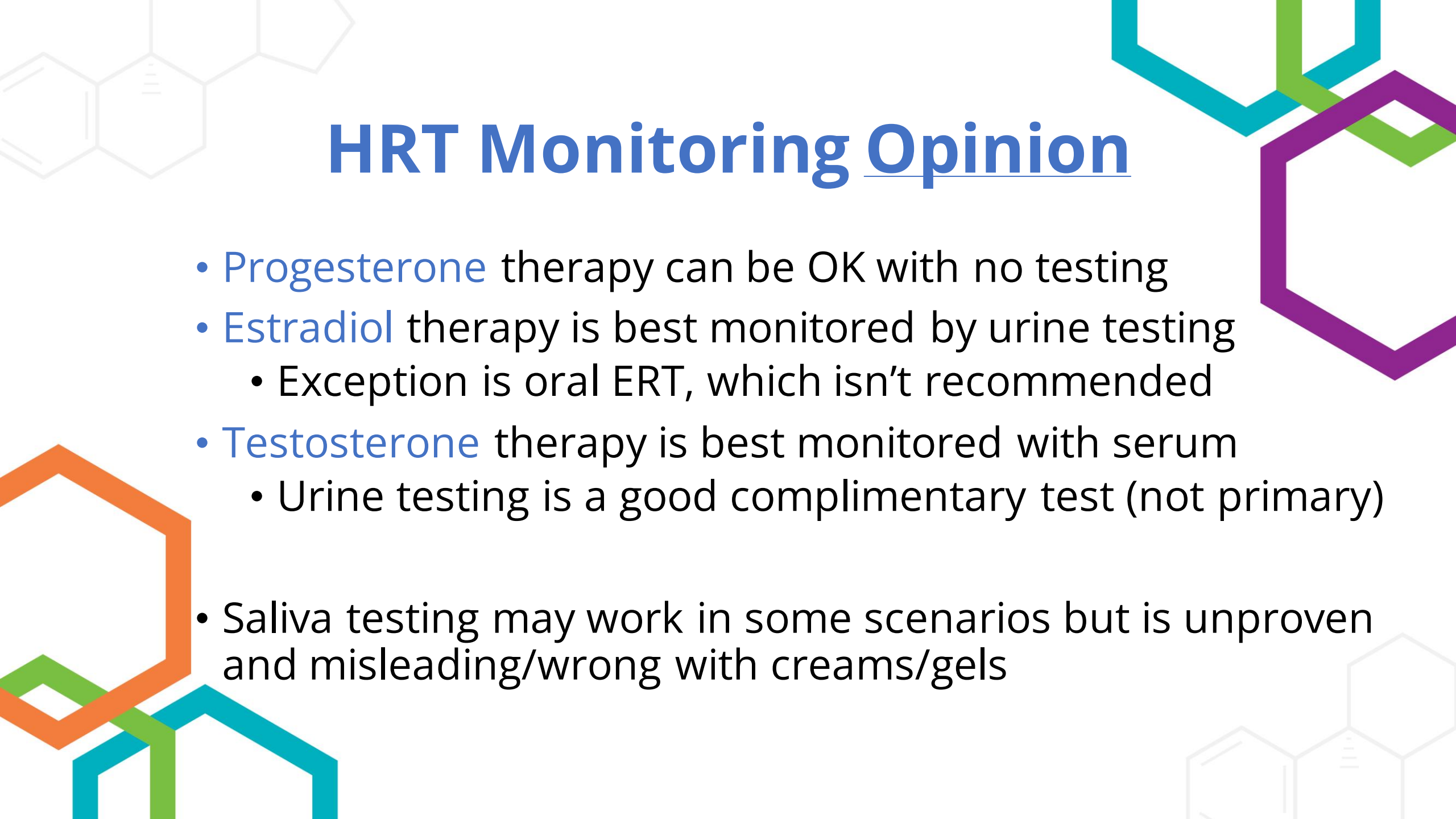
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HRT Monitoring Opinion

- Progesterone therapy can be OK with no testing
- Estradiol therapy is best monitored by urine testing
 - Exception is oral ERT, which isn't recommended
- Testosterone therapy is best monitored with serum
 - Urine testing is a good complimentary test (not primary)
- Saliva testing may work in some scenarios but is unproven and misleading/wrong with creams/gels



Assessment of estrogen exposure from transdermal estradiol gel therapy with a dried urine assay

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Urinary Estradiol

Estradiol

Estrone

Gas Chromatography

Tandem Mass Spectrometry

ABSTRACT

Transdermal estradiol gel is a commonly used menopausal hormone therapy. In research studies investigating the pharmacokinetics and clinical utility of transdermal estradiol gels, serum is often used to measure estradiol levels. Serum results only represent a moment in time during phlebotomy and thus provide little information and allow for limited inference unless serial measurements are performed. In contrast, dried urine may provide a representation of serum estradiol levels over a longer period of time, while also being non-invasive and easier to collect. The primary aim of this study was to evaluate a dried urine method to determine if it may be a viable option for evaluating estrogen exposure resulting from transdermal estradiol gel use. A secondary aim was to explore differences in the urinary estrogen profiles of premenopausal women on no therapy and postmenopausal women who were either on transdermal estradiol gel therapy or no therapy at all. The results of this study demonstrated that the expected dose-proportional changes in estrogen exposure can be observed in the urinary estrogen profile using a GC–MS/MS dried urine assay. The GC–MS/MS assay also showed the differences in the urinary estrogen profiles of premenopausal women, postmenopausal women on estrogen replacement therapy, and postmenopausal women on no therapy.

DUTCH E2 Gel Data published (Steroids 2022)

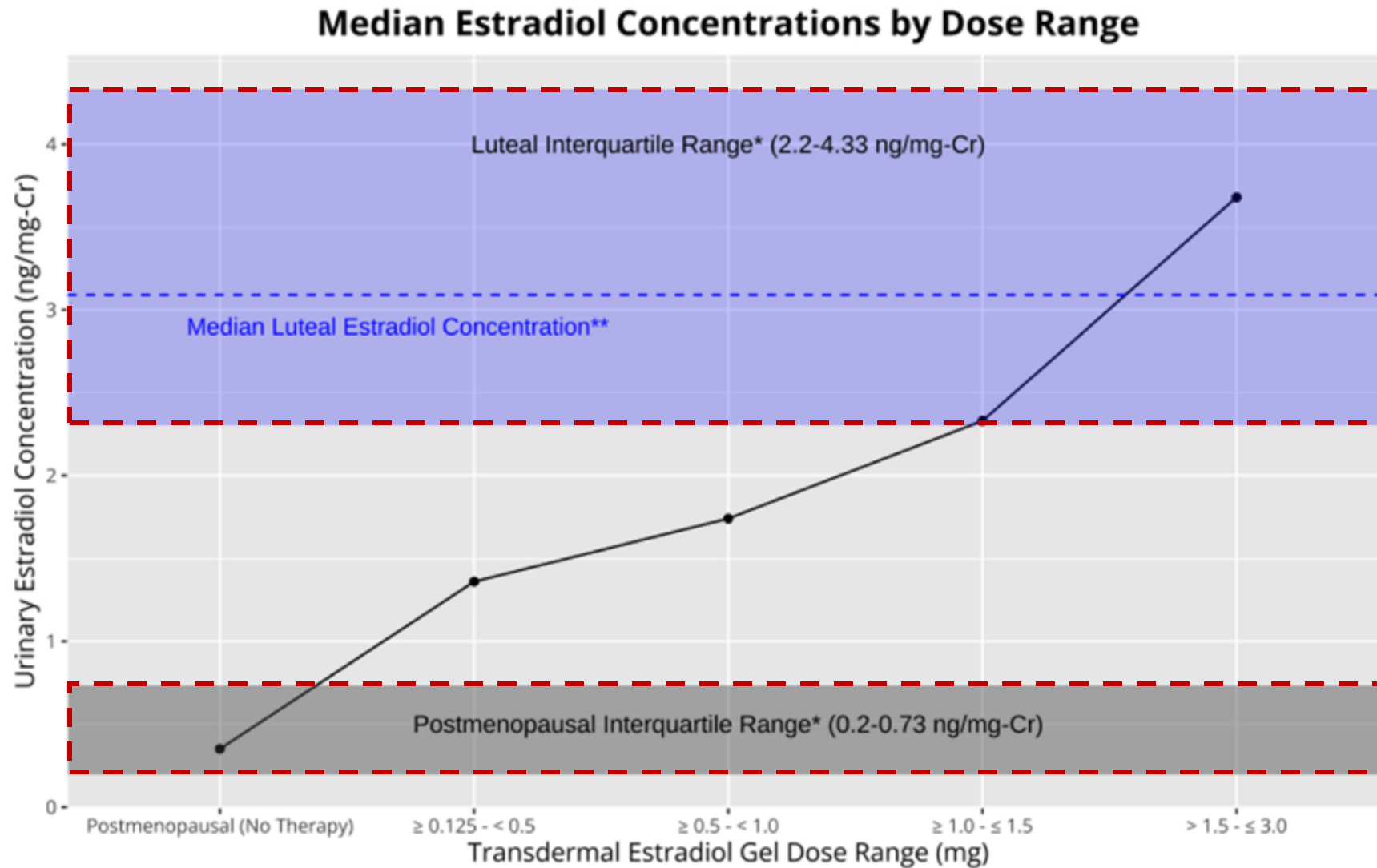


Figure 1.
 $p < 0.001$ for the nonparametric ordered trend (Jonckheere-Terpstra trend test) of concentrations across dose categories.

Fig. 1. $p < 0.001$ for the nonparametric ordered (Jonckheere-Terpstra trend test) of concentrations across dose categories.

Dried Urine

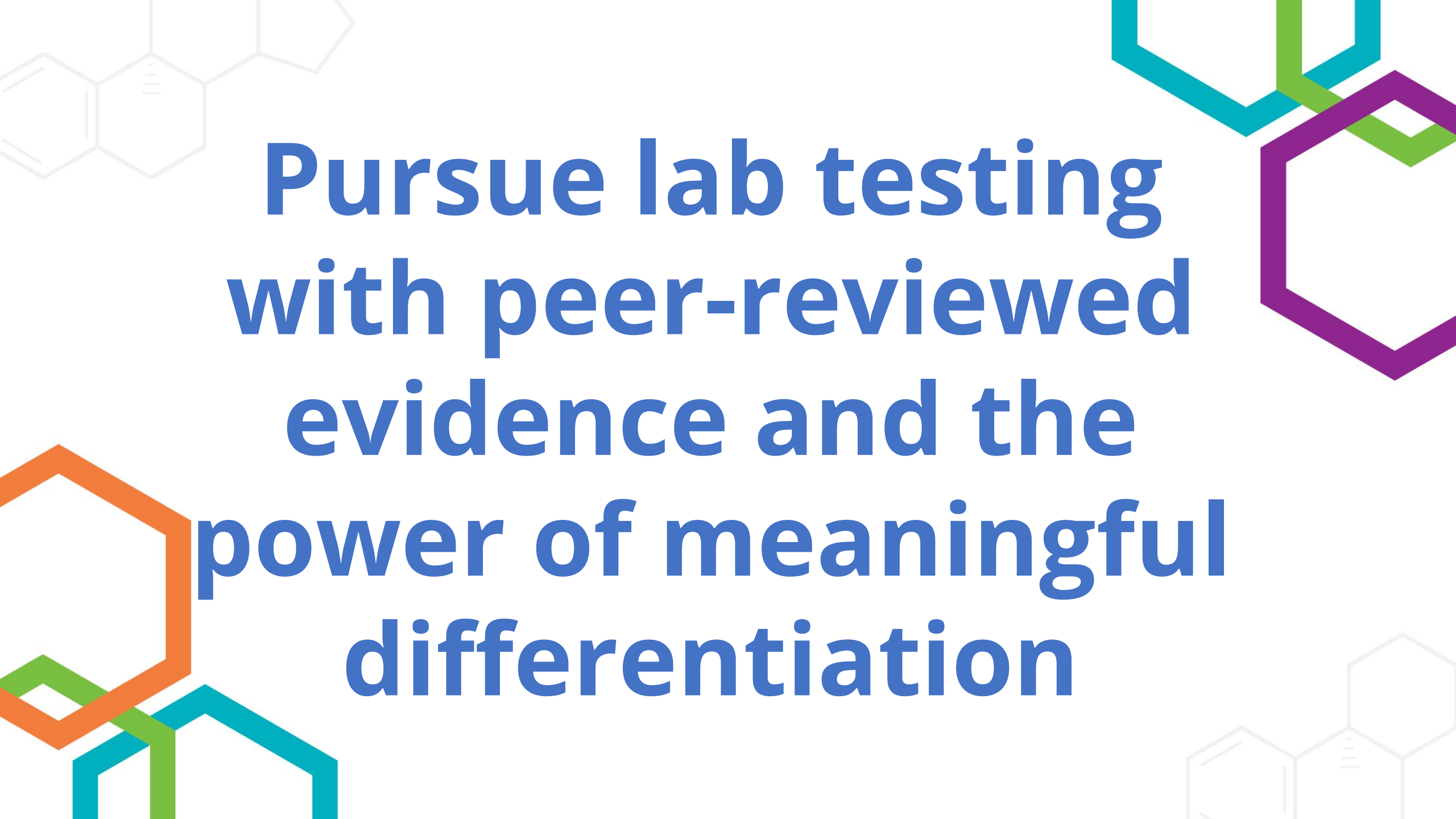
Serum

Saliva

**Be prepared to use these
two in combination for
HRT monitoring**

**Be prepared to use all three strategically to
characterize potential hormone dysfunction**

**Be a lifelong student of the pros and cons of
each, with and without HRT!**

The image features a central text block surrounded by decorative hexagonal patterns in the corners. The top-left and bottom-right corners contain faint, light gray hexagonal outlines. The top-right and bottom-left corners contain bold, colorful hexagonal outlines in teal, green, orange, and purple, some of which are interlocking.

**Pursue lab testing
with peer-reviewed
evidence and the
power of meaningful
differentiation**

Peer-Reviewed Published Validation

- **Urine and Salivary Cortisol, Cortisol Metabolites – J. Clin. Transl. Endoc. (2020)**
 - **Diurnal cortisol from dried urine and saliva correlate** (68 individuals collected both on the same day)
 - Dried urine and liquid urine and 24-hour urine correlation, cortisol and its metabolites
- **Estradiol, Estrone, Progesterone Metabolites – BMC Chemistry (2019)**
 - Dried urine and **serum correlation**
 - Dried urine and liquid urine and 24-hour urine correlation
- **Estrogen & Androgen Metabolites, Melatonin, OATs – BMC Chemistry (2021)**
 - Dried urine and liquid urine and 24-hour urine correlation, estrogen/androgen metabolites
 - Dried urine and liquid urine correlation, organic acids, melatonin
- **Monitoring HRT – Patches, Creams, Gels (2021 NAMS Presentation X3)**
 - **HRT correlation** with dosage and general agreement with reported clinical changes
- **Monitoring Vaginal Estrogen Products, DIM (2022 NAMS Presentation X2)**
 - **HRT correlation** with dosage for vaginal Biestrogen
 - Published changing estrogen metabolite patterns with and without the use of DIM
- **Correlation between DUTCH Androgens and PCOS (2022 ASRM Presentation X3)**
 - **Accurate clinical outcomes correlating elevated androgen metabolites for PCOS patients**
 - Differences in Cycle Map E2 and Pg patterns when comparing ovulating vs non-ovulating women
- **Monitoring Estradiol Gel, Full Manuscript Publication (Steroids 2022)**
 - **Recently accepted! Shows dose-dependent relationship between E2 gel and DUTCH E2 results**

More Coming in 2022

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Thank you!

Questions?

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