Hormone Profiling: Saliva, Blood & Urine

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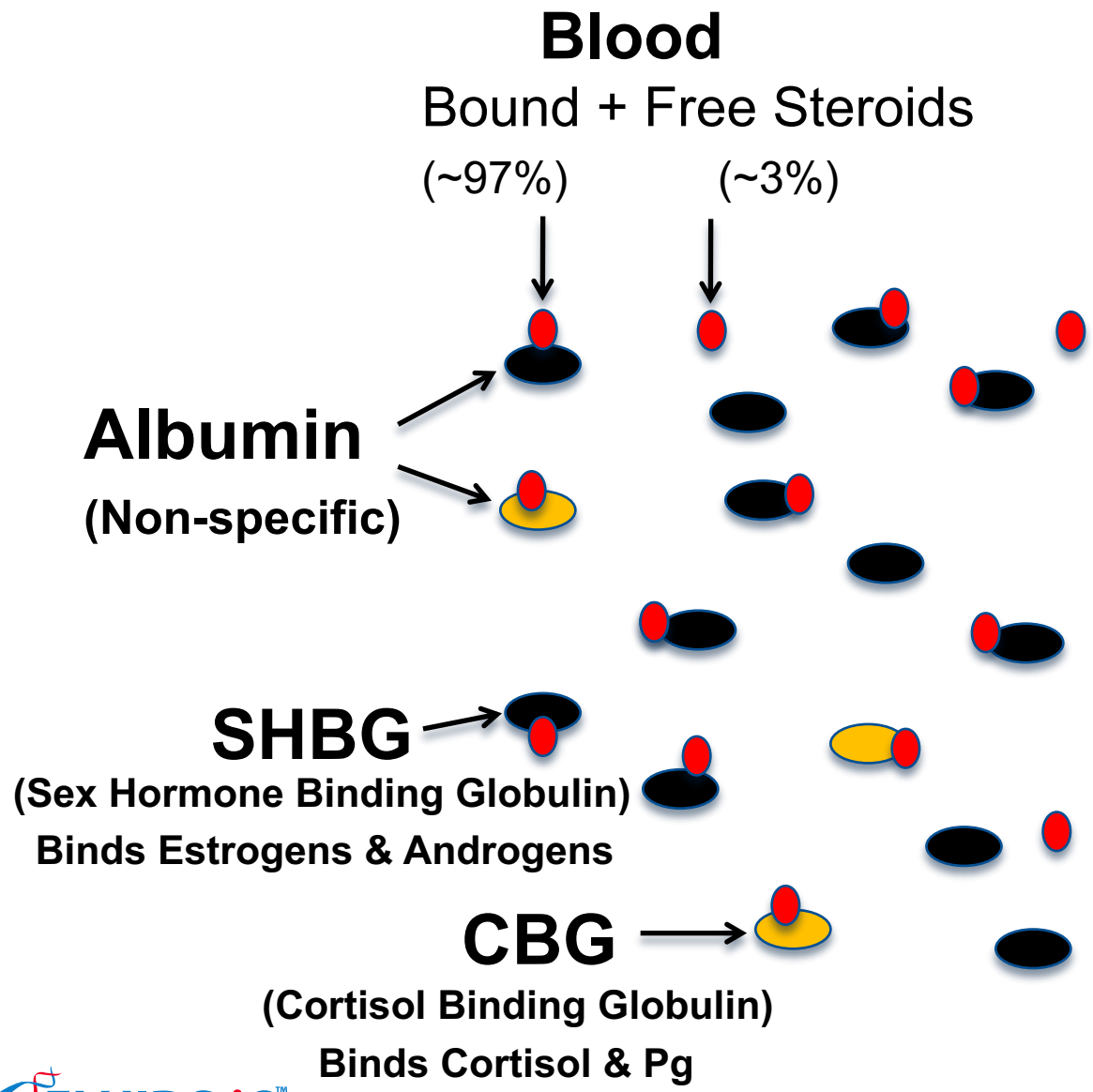
Accurately Assessing Sex and Adrenal Hormones

- Which ‘Medium’ should be used?
- Which is the ‘Best’?
  - What questions need answers?

Consideration of the Relative Merits of the Three Primary Analytical Mediums Used to Test Hormones

Blood (Serum)

Steroid Hormones in Blood



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Serum Sampling

Advantages

- Readily Available
- Cost-effective
- Widely utilized and accepted
- Well-established reference ranges
- Measures SHBG & CBG
- Suitable for testing peptide hormones
  - eg., Prolactin, TSH, FSH, LH:
    Due to their molecular weight, do **not** appear in filtrates, in saliva or urine
  - Mostly reimbursable by insurance

Disadvantages

- **Spot Test:** Provides snapshot in time
  - **Hormone levels time-dependent, with ultradian, circadian & diurnal periodicity**
- **Poor reliability for pulsatile secretion: Major fluctuations, especially in sex hormones**
- Short half-life. Rapid clearance & compartmentalization
- **Generally no distinction between bound & free hormone fractions**
- **Invasive collection**
- Specificity often poor in immunologically based assays; eg., RIA & ELISA
- Full hormone profiling is cost-prohibitive.

SHBG = Sex Hormone Binding Globulin; CBG = Cortisol Binding Globulin

Ultradian = recurrent cycles in a 24-hour day; Circadian = Complete one cycle daily

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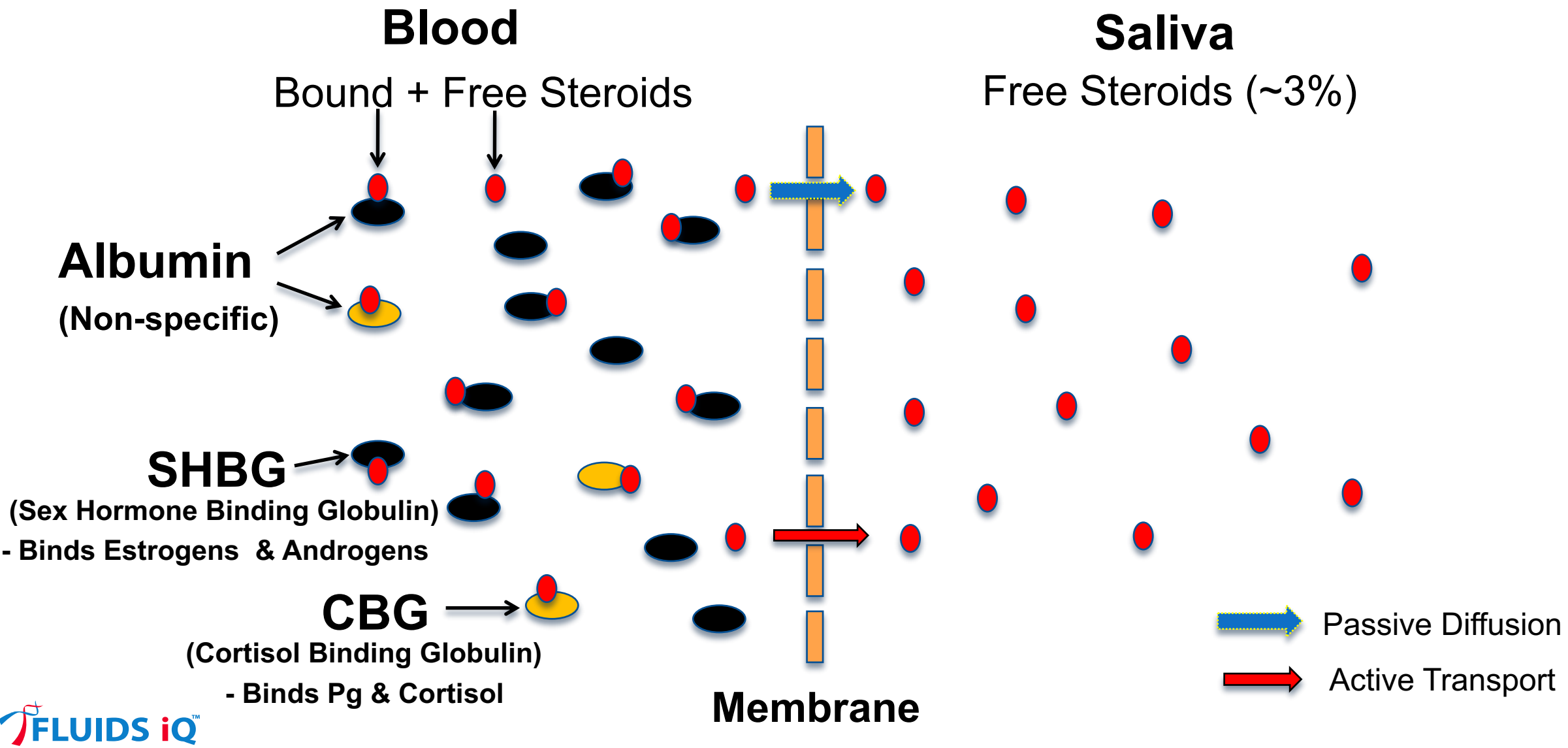
Saliva

Bound vs Free Steroid Fractions



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Saliva Sampling

Advantages

- Excellent for ***Rapid testing***
  - Accurate measure of validated biomarkers
- Well-established reference ranges
- **Non-invasive**, with low infection rate
- Measures **free hormone fraction**
- Ideal for **multiple or serial sampling**
  - More versatility than serum for evaluating un-supplemented hormone status
- Accessible to practitioners, without a license, to order blood tests

Disadvantages

- **Production** difficult for some pts
  - Eating, drinking, gum-chewing, use of make-up
  - Inadequate volume in some > 40 yo (> females)
    - (Sjogren's Syndrome, etc)
  - Micro-damage from tooth brushing (~1hr prior to testing), even with no bleeding signs
- **No measure of hormone metabolites**
- Collection can, itself, significantly alter hormone concentrations

Salivary Hormone Testing

- In bloodstream: Endogenously or exogenously-produced hormones
  - 97–98% of the active hormone are bound up by specific proteins
  - 2–3% released in capillary beds and into interstitial space & tissues

- In saliva - most metabolites from oral hormone therapy filtered out by salivary gland. Only **active** hormones enter saliva
  - ***Salivary hormones more representative of the amount of bioactive steroid present in the bloodstream, & its bioavailability to target tissues***

FLUIDS iQ - Stress and Hormone Profile

Stress and Hormone Profile: Standard & Advanced

A) Standard:

- **Adrenal function, Neuropsychiatric Disorders & Aging:**
  - Diurnal Cortisol, DHEA
- **Sex Hormones:** Estrogen, Progesterone, Testosterone
- **Intestinal Immunity:** Secretory Immunoglobulin (SIgA)
- **Circadian Rhythm & Anti-Oxidant Status:** Melatonin

B) Advanced: Addition of the following

- **Metabolic Function**: Insulin – Fasting & Non-Fasting

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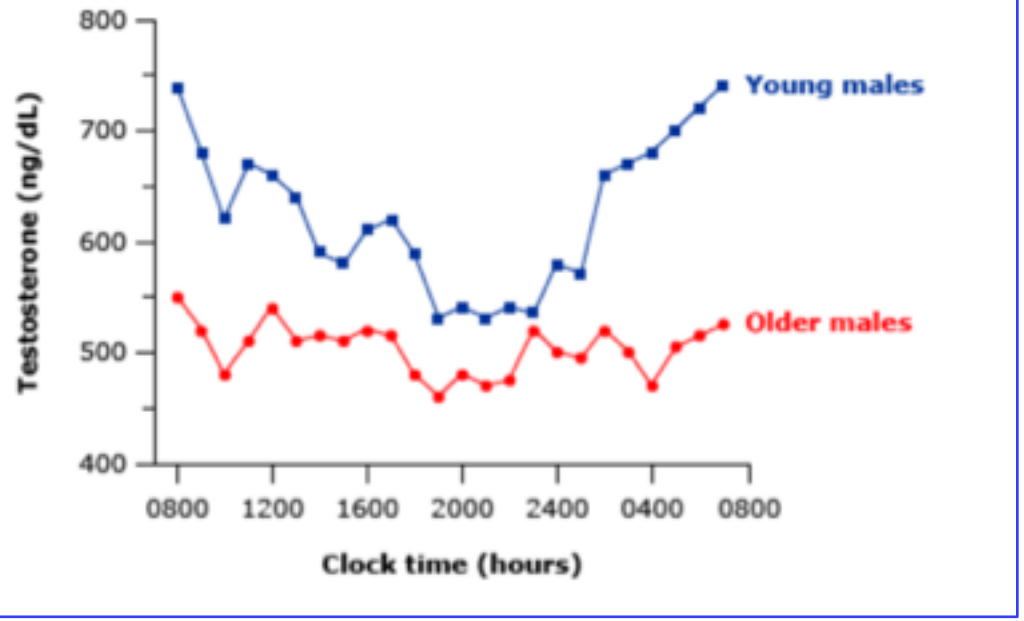
Urine

Diurnal Pattern of Testosterone (T) Secretion



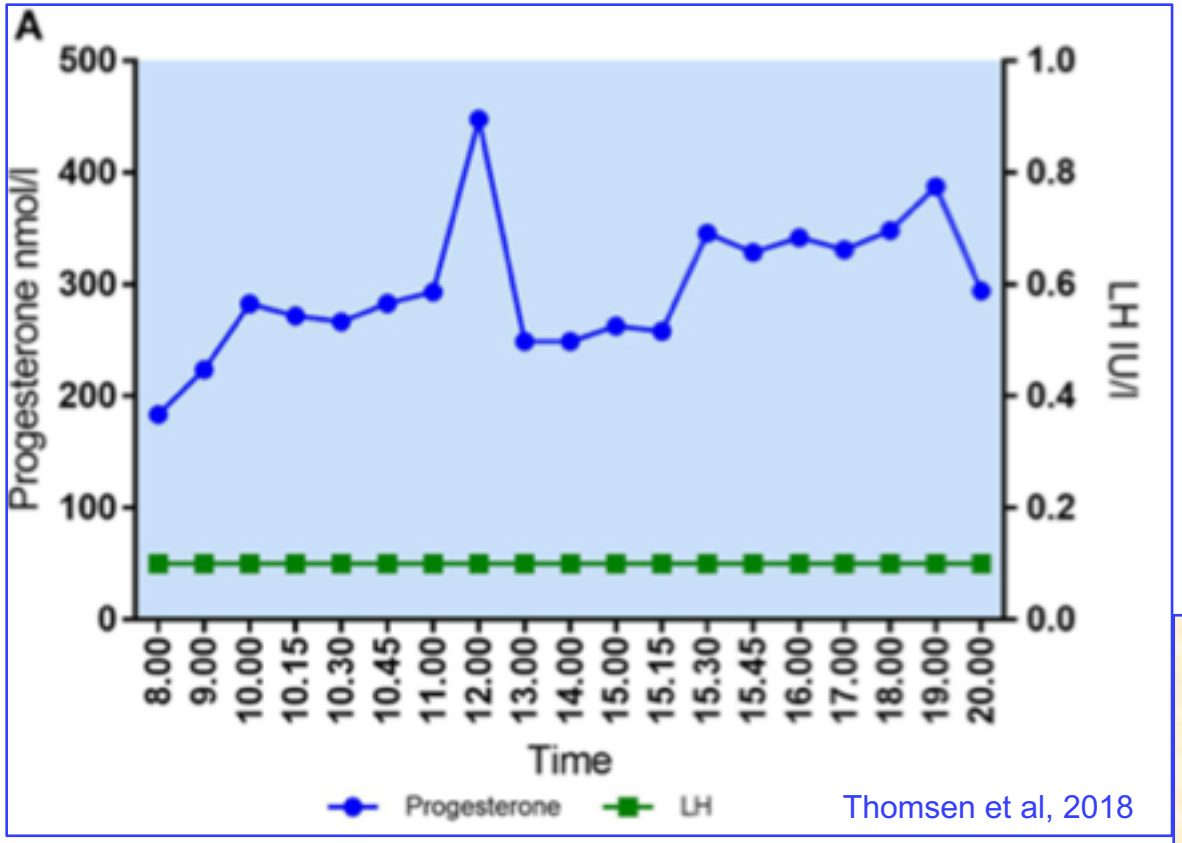
Brenner et al, 1983

- Testosterone levels:
  - Peak at ~ 8 AM (0800), then **fluctuate constantly** throughout the day
  - Lowest at ~ 8 PM (2000), then climb at night
- In 40yr old, AM readings may be 200 points higher than in evening, versus a 50-point difference for a 70 year old

- **Interpretation of results of hormone measurements requires recognition of the biological daytime and day-to-day changes of hormones.** Ahokoski, 1998

Daytime Variation in Serum Progesterone (Pg)

During Mid-Luteal Phase



Thomsen et al, 2018

- Pg released into bloodstream in **pulsations,** leading to large fluctuations in serum Pg.
- Up to **8** fold in 90 min & **2.3- 40.1ng/ml (8-140 nmol/L), or ~ 20** fold change over 24 hrs

Single blood or urine test unreliable

Ideally, test blood or urine over 24 hrs, or 2-3 days in a row, to get average*

*Beckley, 2021

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24 hr Urine Sampling

- **Advantages:**
- Shows **unbound (bio-available)** fraction
- Allows determination of **metabolites**
- Prevents **false** highs & lows as occurs when testing '**spot**' methods like serum, as well as dried urine
- Clinically:
  Correlates well with **symptoms** on hormone questionnaires
  - Allows evaluation of adequacy & safety of **exogenous estrogen supplementation**

- **Disadvantages:**
- Results affected by significant liver or renal impairment, dehydration or excessive fluid intake: **Fundamental Creatinine issue**
- Result misinterpretation possible due to hepatic '1st-pass' effect, if on oral HRT
- **Does not elucidate *diurnal cortisol***
  - Best in salivary testing

Urine Testing & Hormone Supplementation

- Urine testing reveals the ~ **total hormone consumed & eliminated,** but **NOT** how much of the **bioactive & available** hormone is present in the systemic circulation, or has already entered target tissues.
- **Conclusion**: Urine testing an excellent way to evaluate **endogenous** production of the sex hormones, but:

Not clinically useful as a diagnostic fluid for exogenous oral or topical hormone delivery

Dried Urine Spot Sampling

- All samples obtained at a defined moment in time are, by definition:

A SPOT TEST

- A Spot test is a **'snapshot in time'**, which can be useful for certain analytes, but has fundamental flaws & compromises, especially:
- - for pulsatile analytes (eg; hormones)
- - when compared to a full 24-hr hormonal production cycle

- Forced to normalize to the breakdown product of creatine phosphate from muscle and protein metabolism, & found in urine - ***Creatinine***

Dried Urine & Creatinine Normalization

Ideally, want to know how much hormone is produced in the classic µg/24 hrs.
This eliminates urine volume variability (within day & day-to-day)

- Instead, forced to normalize to ***Creatinine (Cr)***, shown as ng of analyte/mg Cr
  - Fundamental problem for **pulsatile** analytes, such as hormones
  - Creatinine levels vary widely with age, sex, diet, exercise, kidney function & genetics
  - Subject ends up as own control. **Cannot be classified with a typical reference range**

- Reference ranges quoted for spot urine samples span an order of magnitude, that is a **factor of 10!**
- In contrast, span for 24-hr urine sample lies between a factor of **2 to 3**

Dried Urine (DU) Spot Tests

- In absence of independently published methods/data & given the limited urine volume in a Dried Urine sample for this type of analysis, it is unclear how quantitation of certain analytes can be **credibly accomplished**, **accurately assessed** and **reproduced** with confidence

The Dried Urine paradigm is arguably outside acceptable scientific standards

Serum Testing for Hormones

- Limited utility for sex hormones, because:
  - No distinction made between **bound & free** hormone.
  - Estradiol, estrone, estriol and Pg reported as **total** hormones
  - **Free** hormone assays not common, leading to misleading results
    - Hormone levels appear to be normal, or even high normal, because of an abundance of **bound** hormone

However, if the free hormone level is low, patient can be functionally deficient, even with a normal total hormone level

Appropriate Body Fluids for Testing Exogenously Delivered Hormones

Type of Fluid	Non Endogenous	Oral Steroids	Topical Steroids	Vaginal Steroids	Troche Steroids	Pellet/IM Steroids
Serum	Yes	Yes[1]	No[2]	No[2]	Yes	Yes
Saliva	Yes	Yes	Yes[3]	Yes	No[4]	Yes
Urine	Yes	Yes[1]	No[2]	No[5]	Yes	Yes
DBS	Yes	Yes[1]	Yes[6]	Yes	Yes	Yes

1. Overestimation: Possible metabolite interference with immunoassays (GC/MS – OK)

Saliva & DBS, but not serum/plasma or urine, are the only way to *accurately* monitor exogenous Topical and Vaginal steroid hormone therapies

6. Overestimation: If hands/fingertips used to apply hormones within 24 hrs of testing

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Zava, 2015

Conclusions: Fluid Mediums For Hormone Testing

For Exogenous Hormones

- **Serum**: For accurate oral administration of sex hormones, needs extraction & separation of metabolites
- **Urine**: Not recommended unless range re-established for dosing (eg,100–300 mg oral Pg) & range readjusted up to reflect the expected level
- **Serum/plasma & Urine**: NOT recommended for testing steroid hormones delivered by topical route of administration.

Best with Saliva

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