



BEST PRACTICES FOR SPECIMEN COLLECTION

SALIVARY HORMONE TESTING

The following collection guidelines are specific to Doctor’s Data reference ranges and vary based on BHRT routes of delivery. Following these intervals assures your patients’ results correlate to the supplementation ranges provided.

Note: For providers interested in testing endogenous/baseline levels of hormones, your patient must avoid hormone use for at least 72 hours prior to collection. After 72 hours, supplementation ranges are no longer applied.

Route of Delivery	Dosage Interval
Topical (including vaginal administration)	12-24 hours prior to sample collection
Sublingual (dissolved under the tongue)	24-36 hours, followed by two 8 oz. glasses of water
Oral	Continue day of collection
IM/SQ Injections	Mid-Cycle
Subcutaneous Pellets	Mid-Cycle
Transdermal Patch	Mid-Cycle
Cortisol	Discontinue 4-5 days prior to sample collection

Topical (including vaginal administration): The ideal dosage interval is 12-24 hours before sample collection (the time between last hormone application and first morning sample collection should be 12-24 hours). DO NOT APPLY ANY TOPICAL HORMONES THROUGHOUT THE ENTIRE DAY OF COLLECTION, RESUMING USE AFTER THE 4TH SAMPLE HAS BEEN COLLECTED.

Note: For dosages typically applied at bed-time, refrain from use the evening prior to sample collection or use topical formulation earlier in the day if the period between application and sample collection will be less than 12 hours; i.e.: 6:00 PM application for a 7:00 AM wake-up/morning sample collection (giving a 13 hour dosage interval).

Sublingual (dissolved under the tongue): The interval between last hormone usage and first AM salivary sample collection should ideally be 24 to 36 hours. After the last dose of the hormone has dissolved in the mouth, it should be followed by two 8 oz glasses of water to clear the mouth of any residual hormone. The first saliva sample should then be collected 24 - 36 hours later. DO NOT USE ANY SUBLINGUAL HORMONES THROUGHOUT THE ENTIRE DAY OF COLLECTION, RESUMING USE AFTER THE 4TH SAMPLE HAS BEEN COLLECTED.

Note: Residual contamination is often observed in samples with dosage intervals under 24 hours.

Oral: It is recommended to continue PO supplementation as prescribed on the day of collection.

IM/SQ Injections: The recommended dosing interval for IM hormones is mid-cycle. For example, the ideal sample collection day for hormone(s) being injected every two weeks (14 days) is 7 days after injection.

Subcutaneous Pellets: The recommended dosing interval for subcutaneous pellets is mid cycle. For example, the ideal sample collection day for hormone level monitoring when pellets are replaced every 90 days, is approximately day 45.

Transdermal Patch: Though the hormone in a transdermal patch is formulated for continual release, a tapering of dose does occur. Therefore, the recommended dosage interval for transdermal patches is mid cycle. For example, if a patch is replaced weekly (every 7 days) ideal sample collection should occur approximately 3-4 days after application.

Cortisol/Glucocorticoid Supplementation: Oral cortisol peaks and returns to baseline within 1-3 hours; therefore it is difficult to measure the therapeutic levels of hydrocortisone/cortisol. It is typically recommended to evaluate endogenous production of cortisol and, as such, recommended to stop ALL cortisol supplementation (including inhalers and topical creams) 4-5 days prior to sample collection.

Melatonin Supplementation: Last dose should be at least 36 hours before first saliva sample collection. recommended to stop ALL cortisol supplementation (including inhalers and topical creams) 4-5 days prior to sample collection.

Health Disclaimer: All information given about health conditions, treatments, products and dosages are not intended to be a substitute for professional medical advice, diagnosis or treatment.



Collection Schedule

Menopausal Status	When to Collect Sample
Premenopausal, regular cycles (28 days)	Days 19-23 Mid luteal phase
Premenopausal, regular cycles longer than 28 days	Count back 7-9 days from usual end of cycle, at a minimum of day 19 Luteal phase is almost always 14-16 days long
Perimenopausal, irregular cycles with ovulation pains	7 days after ovulation
Perimenopausal, irregular cycles, no ovulation pains	Test after day 14, and before day 1 of next cycle
Premenopausal, irregular cycle shorter than 14 days	Days 7-9
When there is no point of reference, e.g. 60 days	Collect samples; freeze; if no period within 2 days then mail the sample
Men and postmenopausal women	Anytime

Schedule	When to Collect Sample
Night shift workers	Collect first sample 30 mins after awakening regardless of time of day. Time of month should follow schedules above. Add note to requisition form noting shift worker.
Frequent travelers (especially across multiple time zones)	If possible, collect samples after 2 weeks at home on regular schedule.

Cortisol levels have a natural diurnal rhythm that varies throughout the day. Cortisol levels should reach their peak approximately 30 minutes after waking and then gradually decline throughout the day. The AM30 cortisol level is the most important, as it gives us the most information about adrenal function.

Always Collect 4 Samples

We request that samples be collected at all four times during the day, regardless of what lab tests have been ordered.

When saliva kits are received by the lab, a fifth pooled tube of saliva is created by collecting a measured sample from each of the four submitted tubes. The fifth tube is mixed thoroughly to provide homogenization and becomes the source from which estrogen, progesterone, testosterone and DHEA are measured. Sex hormone levels fluctuate throughout the day. The pooling of these four samples enables the laboratory to provide a much better reflection of each patient's hormonal status. It is essentially an average of the four submitted samples and more accurately reflects physiologic hormone levels.

Sample Schedule for Follow-Up Testing

At 2-3 Months	Any new patient with supplementation related to E2, E3, PG, T, and DHEA	Comprehensive Plus, Comprehensive Short, or Basic Panel depending on what is being treated
Up to 6 Months	Any patient still reporting symptoms after initial supplementation plan for E2, E3, Pg, T, and DHEA	Comprehensive Plus, Comprehensive, Short, or Basic Panel depending on what is being treated
	Any patient on adrenal support supplementation	Comprehensive, Adrenal, or Cortisol Panel
At 12 Months	All patients	Comp. Plus, Comp., Short, or Basic depending on what is being treated

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Filling out the Hormone Use Survey:

Note: Some pens can bleed through paper, obscuring information entered on the back page. Please use a ball point pen when filling out this document. Fill out all 3 sections. It is important that you make note of any hormone supplementation you are using in section 1. Make note of the last time you used that particular hormone in section 2, and how often you use the hormone in section 3.

Conjugated Estrogens (i.e. Premarin)	Estradiol + Progestin (i.e. birth control pills, HRT)	Progestin only (i.e. minipill, Depo injection, IUD)	Estrone (E1)	Estradiol (E2)	Estriol (E3)	Progesterone (P4)	Testosterone (T)	DHEA	Corticosteroid (i.e. cortisol, hydrocortisone, prednisone)	Pregnenolone	Thyroid	Melatonin
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Conjugated Estrogens - This refers primarily to Premarin (conjugated equine estrogen). It is not referring to bioidentical estradiol or Bi-Est.

Estradiol + Progestin - This column refers to birth control pills, contraceptive rings, or conventional hormone replacement therapy that includes an estrogen and a progestin (synthetic progesterone). If you are taking bioidentical progesterone and estradiol, please do not mark this column.

Progestin only - This column refers to contraceptive options that are progestin only, i.e. hormonal IUDs, Depo injections, or the minipill. If you are taking bioidentical progesterone, do not mark this column.

Estrone (E1) - Rarely prescribed, estrone is a component of tri-est formulations.

Estradiol (E2) - This refers to bioidentical estradiol, one component of Bi-Est. This is usually administered in patches or creams, but is sometimes given orally or via vaginal suppository.

Estriol (E3) - A component of Bi-Est. Typically given in a cream, vaginal suppository, or rarely a pill.

Progesterone (P4) - Mark this column if you take bioidentical progesterone. Progesterone can be administered orally, sublingually, or in a cream. If you are getting a synthetic progestin in a birth control pill, or you use an IUD, do not mark this column.

Testosterone (T) - If you take testosterone of any kind (oral, patch, cream, or injection), mark it here.

DHEA - Fill in if you are using DHEA or 7-keto DHEA.

Corticosteroid - Glucocorticoid medications like hydrocortisone, Prednisone, Dexamethasone, etc. are noted here, as are hydrocortisone creams used for skin conditions. Asthma inhalers can contain corticosteroids, and rarely lip balm.

Pregnanolone - Any pregnanolone containing product should be noted here.

Thyroid - If you take T4, T3, or glandular thyroid, please make note of it here.

Melatonin - Any melatonin containing product can be noted in this column.

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Contamination / Exogenous Exposure

Unexpected hormone elevations are commonly due to either contamination or exogenous exposure. **Contamination of a saliva sample** occurs when the saliva sample or sample collection tube comes in contact with a topical or sublingual hormone supplement. Hormones are prescribed in mg dosages, which are noted as 10^3 . Our sensitive testing equipment is designed to measure salivary hormones, which are in much smaller concentrations. To be specific, the sample is measured in picograms or 10^{12} . There are 1 billion picograms in 1 milligram, so even a trace amount of a milligram sample will cause picogram measurements to be significantly elevated. Patient education on how to prevent contamination is imperative and will prevent the need to retest. Exogenous exposure occurs either when the patient comes into contact with another person's topical hormone products or through the use of a product that contains a hidden hormone. This is also called Transference. Most often the exposure is from a partner or household member who is using BHRT.

Common Causes of Exogenous Exposure

Steroid Creams and Inhalers

Steroid medication use can skew salivary cortisol results. Steroids come in many forms including oral medication and inhalers (i.e. prednisone) and creams (i.e. cortisone). Watch out for steroid ingredients in anti-itch creams and even medicated lip balm. Even if the tester is not personally using a cream, trace amounts of topical cortisone cream from household exposure (children, animals, elderly) may be enough to affect levels. All steroid product use should be avoided for 4-5 days prior to testing.

Beauty, Anti-Aging, and Wild Yam Creams

Anti-aging creams, phytoestrogen creams or wild yam creams may contain, but not report, trace amounts of hormones such as progesterone, estradiol, estriol or DHEA.

Transdermal Creams and Gels

Ideally a transdermal hormone user would have a separate bathroom to confine exposure. As this is not often a realistic expectation, the following precautions should be undertaken when using topical hormone therapy:

1. Do not share bath or hand towels.
2. Especially with testosterone therapy, apply cream/gel with disposable gloves to backs of shoulders in AM before getting dressed. Dispose of gloves in such a manner as they will not be touched by anyone else.
3. Place used clothing into a separate hamper.
4. Do own laundry or provide gloves to the person who does testosterone-exposed laundry (including towels).
5. Shower before bedtime to minimize exposure to partner.
6. Never apply hormone cream right before physical intimacy.
7. Consider an alternate mode of hormone delivery if there are children or pregnant women in the household.

Skin to Skin contact

Hands-on practitioners such as massage therapists, chiropractors and acupuncturists can be exposed to their patients' topical creams. Practitioners using hormone creams themselves can also expose their clients to these creams. This is another reason to wash hands thoroughly before collecting saliva for testing.

Gym Equipment

Gym equipment is a common vector of topical hormone contamination. Gym users who use topical hormones on exposed areas should wipe down equipment after use. Everyone should wash hands after visiting the gym.

Prevent Transference and Contamination

Wash Hands

Wash hands thoroughly after applying topical BHRT cream or gel, otherwise residual cream can be found on doorknobs, faucet handles, counter tops, telephones, gym equipment, sheets and pillow cases, etc. This residual hormone can be spread to others via these surfaces (especially important to note in cases of topical testosterone supplementation), and can find its way back onto the hands, face or lips of the patient and subsequently contaminate a saliva sample. For this reason, avoid touching the lips after contact with topical BHRT preparations.

Account for Sublingual Hormones

Sublingual BHRT formulas (troches) are designed to be absorbed by the blood vessels beneath the tongue. While this is an effective way to administer hormones, this route of administration can make it difficult to monitor therapeutic hormone levels. If any of the hormone supplement is still present in the mouth, or saturated in the buccal tissues at the time of saliva collection, contamination will occur. For this reason, we recommend drinking two 8 oz glasses of water immediately after the hormone supplement has completely dissolved under the tongue. The saliva sample should be collected 24-36 hours following this process. Note that sublingual hormones can collect on toothbrushes. To avoid this, brush teeth prior to dosing sublingual hormones.

Clean Contaminated Surfaces

If there is any chance any surfaces were touched after applying a hormone cream or gel, clean the area thoroughly. Wipe down faucets, doorknobs, counter tops, light switches, and soap dispensers with hot soapy water. Phones and remote controls can be cleaned with rubbing alcohol applied first to a microfiber cloth. Change sheets and pillow cases following the last hormone application prior to saliva sample collection.

Wash hands thoroughly prior to touching the sample collection tube or collection straw.

When applying topical BHRT preparations or using sublingual hormones, sample contamination can be easily avoided when the aforementioned precautions are mindfully taken into account.