WHITE PAPER

THE IMPORTANCE OF **ORAL HORMONE SCREENING**

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EXECUTIVE SUMMARY

The balance of your hormone levels has a significant impact on your daily well-being. Even minor disruptions in hormonal function can contribute to weight gain, heightened fatigue, fluctuations in mood, and disruptions in sleep patterns. Hormones levels are constantly changing in your body depending on the time of day, stress, health, and sleep cycle. Despite the importance of hormones, the inconvenience of requiring multiple blood draws and fasting collections prevents many from regular testing. Salivary testing is a non-invasive method for assessing free and active hormone levels that can be conveniently preformed at home.

- The US FDA has found numerous saliva tests for the measurement of free steroidal hormones equivalent to serum-based tests. [1,2,3,4]
- Levels of free hormones in saliva often provide better predictions of health outcomes and physiological effects compared to the total hormone levels in blood.[5]

- Spectrum's specimen preservation solution prevents degradation of hormones in the samples for weeks at room temperature, kills bacteria, inactivates viruses, and ensures testing accuracy.
- The limit of detection (LOD) for the SimplyTest® Cortisol assay is validated to be less than 1 ng/ml of cortisol, effectively 10 times better than many FDA cleared tests. [6]
- The LOD for the SimplyTest® Testosterone assay is 10.3 pg/ml of testosterone, which is better than many FDA cleared serum-based tests.

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HORMONES IN SALIVA

Most hormones in your body are in the blood where they are bound by proteins. [7] When they are in this state they are inactive and don't impact your cells. [8] Only the unbound or free, hormones are able to effect the body. For example less than 2% of testosterone and 10% of cortisol is free in the body at any moment. [9]

Free hormones can leave the blood and diffuse into other body fluids, such as saliva, where they can be measured. Salivary testing offers several potential advantages, including ease of sample collection, the ability to assess diurnal hormone patterns, and the measurement of free, bioavailable hormone levels. Hormone levels in saliva is an accepted tool to by the FDA to predict ovulation based upon natural hormone variations over the menstrual cycle. [10,11]

Measurements from saliva are best utilized when the free levels of hormone are the most important consideration. In many contexts, the levels of free hormones, such as those found in saliva, provide better predictions of health outcomes and physiological effects compared to the total hormone levels, such as is measured in blood. [12]

When further testing is required for diagnosis of specific hormonal disorders, follow-up measurements from blood can serve as a complimentary test. [13]

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SALIVARY COLLECTION

At the time of collection, care must be taken to produce accurate and reproducible results. Critical criteria include ensuring sample stability, consistency in collection, and avoiding contamination. [14]

Spectrum Solutions has extensive experience in standardizing sample collections and innovative assay technology to produce a highly sensitive and reliable laboratory developed test for hormones in saliva. The company's multi-omic collection device (SMD) allows for the quick, simple collection of salivary samples anywhere. The one milliliter sample collection volume means the full sample can be collected and preserved and ready to ship in less than 2 minutes.

The preservation solution prevents degradation of hormones in the samples for weeks at room temperature, kills bacteria, inactivates viruses, and reduces the saliva's viscosity to make accurate analysis easier. This provides ample time for the sample to arrive at the laboratory. Using specialized monoclonal antibodies in enzyme-linked immunosorbent assays our testing produces accurate, highly sensitive, and reproducible measurements for hormones. The monoclonal antibodies used for these hormone assays are obtained through FDA cleared sources.

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SALIVARY CORTISOL

Cortisol is a stress hormone that plays a crucial role in the body's response to stress and your daily rhythms. Salivary cortisol levels provide insights into an individual's stress response and their ability to regulate stress.

Salivary cortisol testing is employed to monitor the efficacy of interventions targeting cortisol regulation, such as stress reduction techniques, behavioral therapies, pharmacological treatments, or identify certain endocrine disorders.

In addition, cortisol levels naturally fluctuate throughout the day, following a diurnal rhythm, which can affect sleep patterns; testing enables the assessment of this rhythm by collecting multiple samples at different times and provide insights into an individual's overall wellness.

The SimplyTest Cortisol test uses multiple samples taken throughout the day at specific times to assess an personal cortisol profile. The limit of detection (LOD) for the SimplyTest® salivary cortisol assay is validated to be less than 1 ng/ml of cortisol, effectively 10 times better than many FDA cleared tests.15 In the SMD preservation solution, salivary cortisol remains highly stable after repeated freeze thaw cycles (3x), high temperature excursions (40° C and 104° F), and for 22 days of at room temperature (see Figure 1).

After 14 days saliva without preservative was no longer able to be tested due to bacterial growth, and blood specimens requiring cold storage to maintain stability. Testing is highly specific and selective for Cortisol with minimal cross reactivity (see Table 1). [16]

Figure 1: Cortisol Stability in saliva multi-omic device

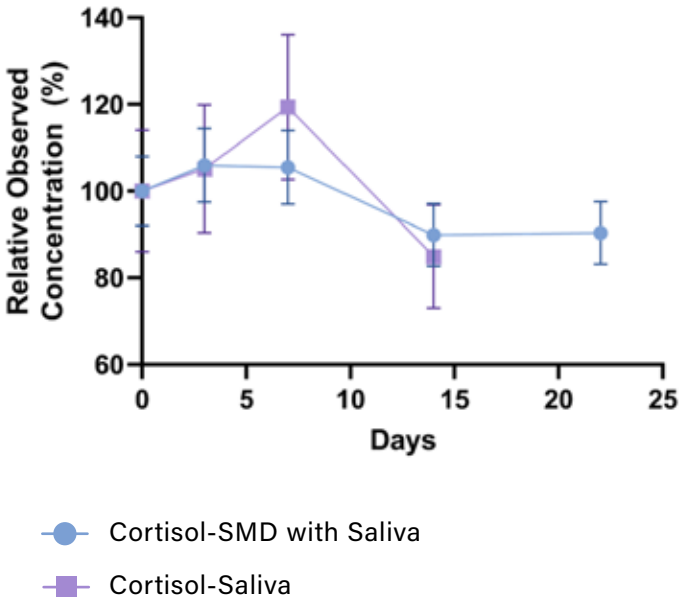

Table 1: Cortisol Assay Cross Reactivity

Interfering Agent	Concentration (ng/mL)	%Cross-reactivity
11-Deoxycortisol	1.00	ND
DHEA-S	1.00	ND
DHEA	1.00	ND
Estriol	40.00	ND
Progesterone	1.00	ND
Corticosterone	0.80	ND
Testosterone	0.50	ND
Androstenedione	0.36	ND
17β-Estradiol	0.40	ND
17α-Hydroxyprogesterone	0.24	ND
Estrone	0.25	ND
Aldosterone	0.08	ND

ND = not detected.

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SALIVARY TESTOSTERONE

Salivary testosterone testing allows for the assessment of free testosterone. While testosterone is primarily a male sex hormone, it has important physiological functions in both men and women. Abnormal testosterone levels in males can cause low libido, erectile dysfunction, decreased muscle mass, and fatigue. Female symptoms include irregular menstrual cycles, reduced sex drive, and vaginal dryness. [17]

Free testosterone in saliva helps identify testosterone deficiency (hypogonadism) or excess (hyperandrogenism) and is convenient for monitoring the effectiveness of testosterone replacement therapy (TRT) or other hormone boosting interventions. Research has found significant correlations were found between salivary and serum free testosterone (r=0.97) and salivary and serum total testosterone concentrations (r=0.70–0.87). [18,19] In situations where free serum testosterone is the primary consideration, saliva and blood testing can be comparable for making clinical decisions.

The SimplyTest Testosterone assay is a sensitive and specific test for free testosterone. The SMD preservation solution improves the reliability of testosterone collection by stabilizing the sample and facilitating laboratory processing through reduced sample viscosity. While blood specimens require refrigeration 8hrs post-collection, the stability of salivary testosterone was maintained over a seven day period at room temperature in combination with 3x high temperature (40° C) or 3x low temperature excursions (-18° C) (see Figure 2). The limit of detection for the assay was 10.3 pg/ml of testosterone, lower than many FDA cleared serum based testosterone assays. Testing is highly specific and selective for free testosterone with minimal cross reactivity (see Table 2). The assay can also be used under the guidance of a healthcare provider when assessing androgenic hormonal levels in me and in the diagnosis and treatment of hirsutism, polycystic ovarian syndrome, and breast cancer in women. [20,21,22,23,24]

Figure 2: Testosterone Stability in Saliva Multi-omic Buffer after repeated high and low temperature excursions

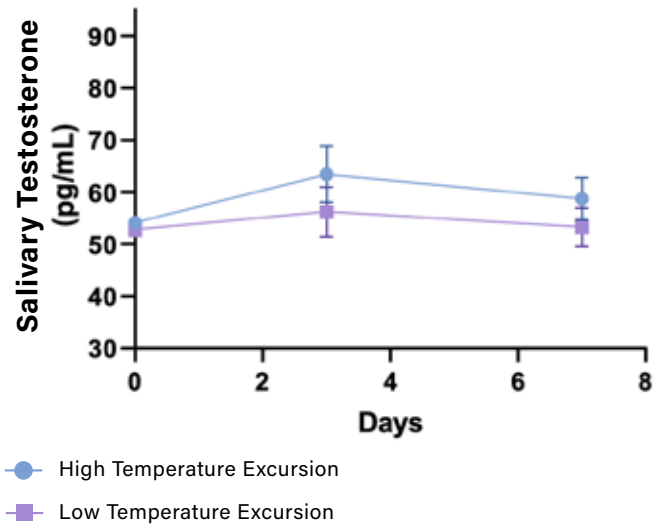

Table 2: Testosterone Assay Cross Reactivity

Interfering Agent	Concentration (pg/mL)	% Cross-reactivity
11-Deoxycortisol	300,000.0	DET
5α-Dihydrotestosterone (DHT)	30,000.0	1.9%
Dehydroepiandrosterone (DHEA)	4,899.1	0.4%
Estriol (E3)	300,000.0	0.0%
Progesterone	20,000.0	0.1%
Corticosterone	4,000.0	0.7%
Hydrocortisone	300,000.0	DET
Androstenedione	1,804.0	1.5%
17β-Estradiol (E2)	1,201.2	DET
17α-Hydroxyprogesterone	1,190.0	DET
Estrone (E1)	748.9	DET
Aldosterone	400.0	DET

DET = Detected but value is < LoQ; ND = not detected.

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CONCLUSION

Saliva has become universally accepted as a sample type for measuring free hormones. Free hormones in saliva, which are not bound to carrier proteins, are believed to reflect the biologically active fraction of hormones available to impact the body. Salivary hormone testing offers a non-invasive and convenient method for: 1) assessing free hormone levels, 2) allowing evaluation of hormone patterns over time, 3) studying stress responses, 4) monitoring hormone replacement therapy, and 5) investigating hormone-related health outcomes. Saliva testing for free hormones has become a valuable tool recognized by the FDA and clinicians.

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