

INNOVATIVE SALIVA.



 **TECAN.**

SPECIALLY DEVELOPED FOR SALIVA.

Steroid hormone assessment from saliva allows the specific determination of the biologically active or “free” fraction of target hormones. Benefit from highly sensitive luminescence immunoassays and ELISAs which have been specially developed and validated for saliva.

All about saliva.

Working with saliva has many advantages: sample collection is non-invasive, painless and stress-free. Multiple specimens may be collected anytime, anywhere, with no need for medical staff. The collection of saliva is simple and allows children and elderly to manage it themselves after a short introduction. Saliva sampling can be performed during physical exercise and rest periods, so athletes can be monitored during training.

A special ultrapure polypropylene sampling device called SaliCap, ensures excellent recovery of progesterone in the saliva.

Proficiency testing.

Meet the first company to implement a quality control system for salivary steroid measurement. We are happy to invite any laboratories working with saliva samples to participate in the proficiency testing service, free of charge! In this way we intend to provide a foundation for inter-laboratory consistency in saliva testing.

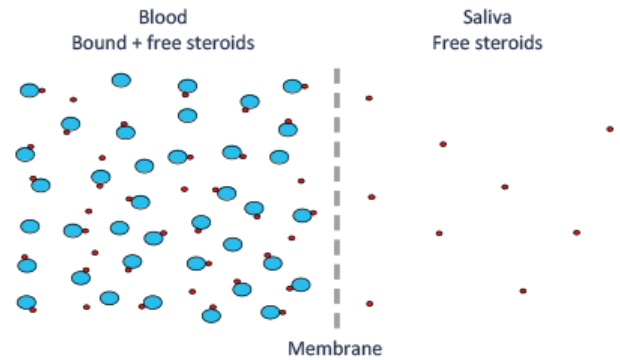


Figure 1: Diffusion of free steroid hormones from blood into saliva

Free and protein bound steroid hormones in blood and free hormones in saliva. The concentration of free hormones is the same on both sides of the membrane.

STERIODS IN BLOOD AND SALIVA.

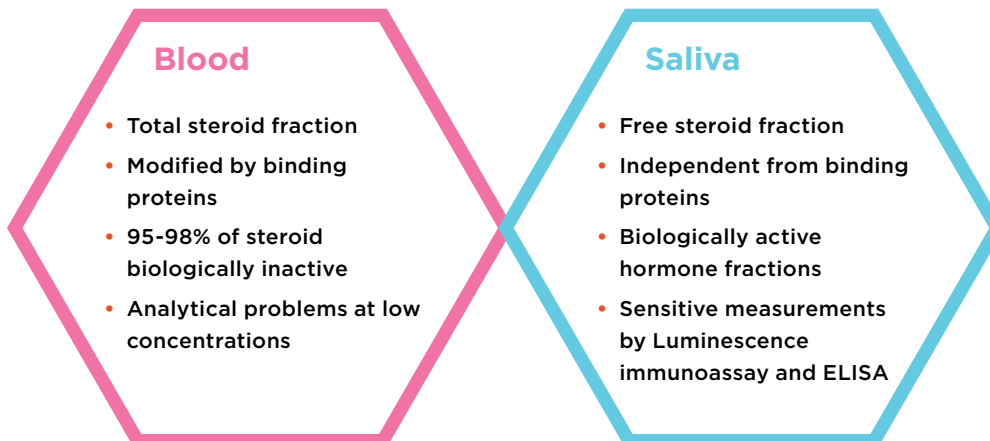


Figure 2: The need to measure biologically active hormone is aided by the fact that only free, unbound hormones leave the bloodstream via the membranes and into saliva. Steroid hormones can cross the blood – saliva membrane via different mechanisms, including expression and secretion, active transport, and passive diffusion (Gröschl, 2008).

STEROID PATHWAY

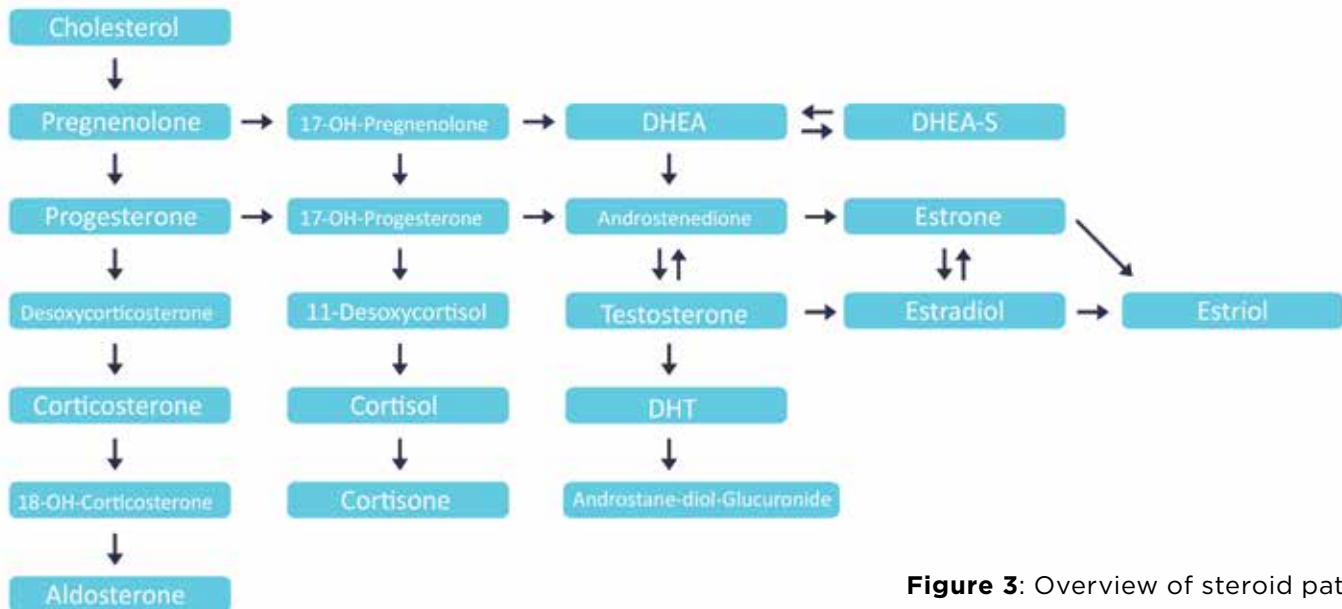


Figure 3: Overview of steroid pathway.

Applications for salivary testing.

Saliva is the specimen of choice in a variety of traditional medical care situations as well as in the emerging areas of health measurement and monitoring. With the ability to evaluate a pooled saliva sample from multiple collections it is possible to get a reliable assessment of hormone concentration even in cases of marked diurnal fluctuation.

Applications

- Psychology
- Occupational medicine
- Endocrinology
- Anti-aging medicine
- Neuroendocrinology
- Oral medicine
- Sports medicine
- Immunology
- Research
- Veterinary medicine

BROAD LEVEL OF APPLICATIONS. ENDOCRINOLOGY.

Androgen Status.

Free Testosterone is an ideal parameter to measure androgen levels in both men and women, but accurate measurement of testosterone remains a challenge. The determination of the blood testosterone concentrations in serum by conventional immunoassays is inaccurate in men, and even more so in women and children. Directly measuring testosterone in saliva is a better alternative to blood measurement: only the free, biologically active fraction is represented in saliva.

The Tecan luminescence immunoassay shows high analytical and functional sensitivity, and allows quantification of the very low concentration in saliva, as well as in diluted serum.

Studies show that free salivary testosterone levels in morning samples correlate well with calculated free testosterone in blood, both in healthy men and in patients with androgen deficiency.

Cushing's Syndrome.

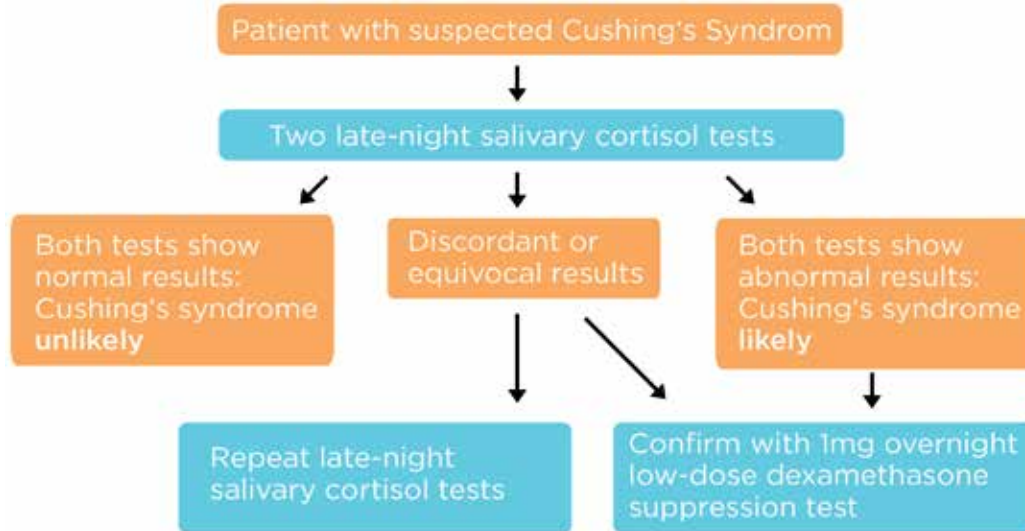


Figure 4: According to Raff H, 2012.

Cushing's Syndrome, for example, results from excessive secretion of one or all of the adrenocortical hormones, mainly cortisol. This disease is characterized by failure to reach the circadian nadir in cortisol secretion late at night. This is quite a difficult endocrine pathology to determine, because of the very common phenotype as seen in obesity, hypertension, depression, alcoholism, etc. Furthermore it is relatively unusual, though growing increasingly common.

Late-night salivary cortisol is now a well-accepted and recommended screening test for Cushing's syndrome. This method shows high specificity and sensitivity, and ELISA or luminescence assays are very easy to handle.

The Tecan Cortisol salivary luminescence immunoassay only requires 20µL of saliva, making it convenient for patients like children or the elderly.

Sports medicine.

During physical exercise, free salivary cortisol, DHEA and testosterone concentrations all rise with the intensity of the exercise.

It is particularly important in sports medicine to note that hormone levels and exercise-induced hormone changes vary among subjects with different types of training. Monitoring hormone levels during sport sessions is found to be a useful

research tool for optimizing athletes' training status and identifying any excessive exercise liable to result in overtraining syndrome.

Measurement of IgA in saliva might provide useful information to athletes.

Anti-Aging.

As a rule, levels of most hormones are decreasing with age. This can be explained by general decline of endocrine function and less sensitivity of hormone receptors with the age.

Thus, in women, decreasing estrogen levels are connected to menopause. In men, testosterone levels significantly decline with aging.

A proper hormonal balance of testosterone, progesterone, and estradiol (among others) is a key to good health. As it was mentioned above, free testosterone can be an ideal diagnostic tool for androgen level, both in men and women.

Measurement of free testosterone is recommended for males with primary and secondary hypogonadism, as well as for women suffering from hirsutism or virilisation.

Hormone replacement therapies may readily be adjusted by assessing salivary hormones.

STRESS AND SLEEP RESEARCH.

Psychological research is related to the science of behavior and mental processes; it includes areas such as biological, cognitive and social psychology. Occupational medicine is the branch of clinical medicine most actively involved in occupational health.

Sleep disorders.

Occupational medicine covers sleep disorders, occurring mostly in shift workers and leading to psychological and physical stress.

In this kind of disorder it is helpful to evaluate individual circadian rhythms, combining measurements of melatonin and cortisol concentration: both hormones are produced according to a circadian rhythm, with contrary circadian patterns.

Changes in the levels of melatonin and alternation in the pattern of secretion have been reported to coincide with: sleep quality, insomnia, daytime sleepiness or jet lag.

Normal cortisol concentration in human saliva during the day is highly dynamic. Cortisol typically peaks in the early morning: the timing of this cortisol peak does not depend on clock time nor is it influenced by daylight: it is set by the individual's time of waking.

Both hormones are regarded as stress markers. Recent studies have shown that melatonin can influence cortisol levels (Rahman, et al., 2019.). These results are useful in the research of sleep disorders.

Burnout.

Recent stress research indicates that more and more people are concerned about work-related stress; this makes occupational stress as important for companies as workplace accidents. The World Health Organization (WHO) has described stress as "one of the major threats of the 21st century". For this reason more and more institutes and physicians are involved in identifying and measuring biological parameters which can reveal individuals' psychobiological and physiological status.

Please, visit our homepage www.tecan.com/ibl for more details of assays of other biological fluids besides saliva, or contact us directly for more information.

Stress research.

Stress research in clinical psychology very often relies on questionnaires, but psychologists are always looking for biological parameters; free salivary cortisol is a very well established, non-invasive marker for hypothalamus pituitary adrenal (HPA) axis activity (internal studies; Wittchen, Schönfeld et al, 2012).

Furthermore, hair cortisol analysis provides a valuable and sensitive retrospective measure of cumulative cortisol secretion over prolonged periods of time (Stalder *et al.*, 2012).

The parameter alpha amylase exhibits relationships with chronic stress, post-traumatic stress disorder, behavior, cognitive functions, and health.

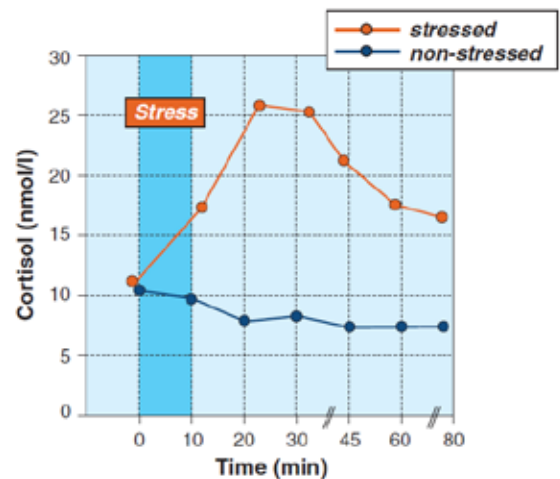


Figure 5: Cortisol responses to moderate psychosocial stress.

SLEEP RESEARCH

Melatonin (N-acetyl-5-methoxy-tryptamine) is synthesized from the amino acid tryptophan and is one of the major hormones of the pineal gland (Fink et al., 2012). There is a circadian rhythm associated with melatonin, with the highest levels in plasma being observed at night (Klein, 2007).

Changes in the levels of melatonin and alternation in the pattern of secretion have been reported to coincide with: sleep quality (Jockovich et al., 2000), insomnia (Almeida Montes et al., 2003), daytime sleepiness (Rose and Kahan, 2001), jetlag (Arendt et al., 1987), depression (Beck-Friis et al., 1985), oxidative stress (Miller et al., 2013), pregnancy (Tamura et al., 2008) and blindness (Sack et al., 1992).

Regulation of melatonin secretion is influenced by light via a pathway that starts in the retina of the eye. The production of melatonin is stopped abruptly upon exposure to light (Fink et al., 2012).

Levels of melatonin in plasma and saliva are correlated throughout the circadian rhythm, with the concentration of melatonin in saliva corresponding to about one third of the concentration in serum in average (Voultsios et al., 1997). A schematic representation of the changes in melatonin concentration over 24 hours is shown in **Figure 6**.

Melatonin concentrations in circulation are highly variable. During the day, melatonin levels are very low, rising. Illustrative examples for melatonin concentrations can be found in the scientific literature: for example in publications by Ba-Ali et al, 2019.

Melatonin concentrations play an important role in the regulation of sleep patterns. The start of melatonin production in the human body can be assessed via the dim light melatonin onset (DLMO), which allows characterization of the circadian rhythm secretion in multiple matrices (plasma, urine, saliva) (Wyatt et al., 2006).

Sleep disturbances in shift workers -can lead to psychological and physical stress. It is helpful to evaluate individual circadian rhythms, combining measurements of melatonin and cortisol

concentrations, which are produced according to circadian rhythms with contrary patterns.

Melatonin is the key biomolecule for promoting nighttime sleep. The melatonin levels increases soon after the onset of darkness, peak in the middle of the night between 2 and 4 a.m. and gradually fall during the second half of the night. The melatonin levels during the day are about 10 times lower.

Cortisol levels, on the other hand, are highly dynamic during the day, and typically peak in the early morning. However, the timing of this cortisol peak depends on the individual's waking time, and not on actual time or daylight. Both cortisol and melatonin are regarded as stress markers. Recent studies have shown that melatonin can influence cortisol levels

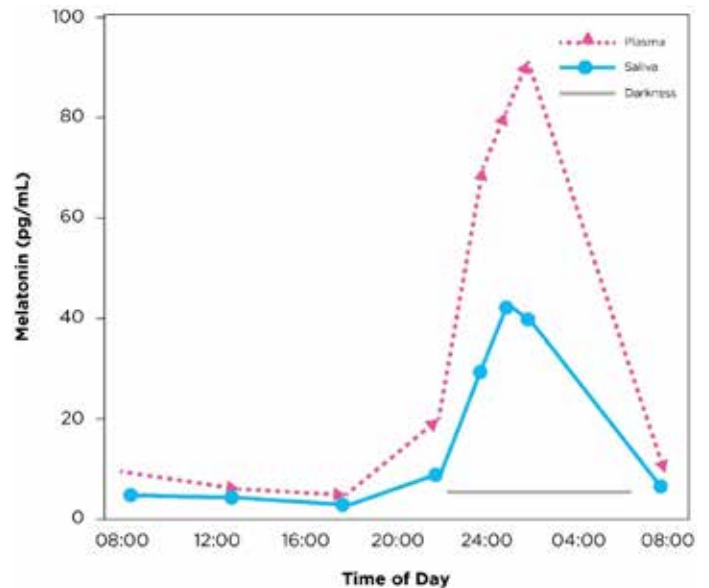


Figure 6: Schematic representation of increase and decrease of melatonin concentration in plasma and saliva over a 24-hour period (Grivas and Savvidou, 2007).

(Rahman, Shadab A., et al., 2019). These results are useful in the testing of sleep disorders.

In apparently healthy individuals, DLMO occurs 2-3h before bedtime. Individuals with sleep disruption or individuals who are involved in shift work (and who therefore cannot maintain a sleep-wake cycle that is coherent with natural daylight and darkness) are affected by phase advances

or phase delays of the DLMO. This is also true for individuals who are exposed to light at night.

Disruption to healthy sleep patterns (exposure to bright light at night-time or the participation in night shift work) can alter the DLMO and lead to a phase advance or phase delay of the melatonin concentration in the sample.

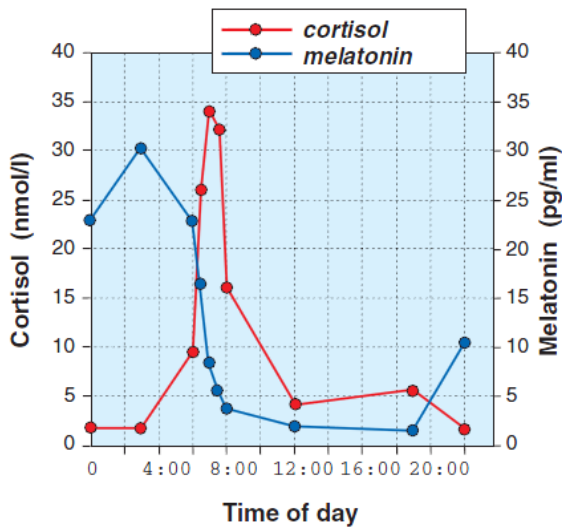


Figure 7: Melatonin and cortisol diurnal pattern.

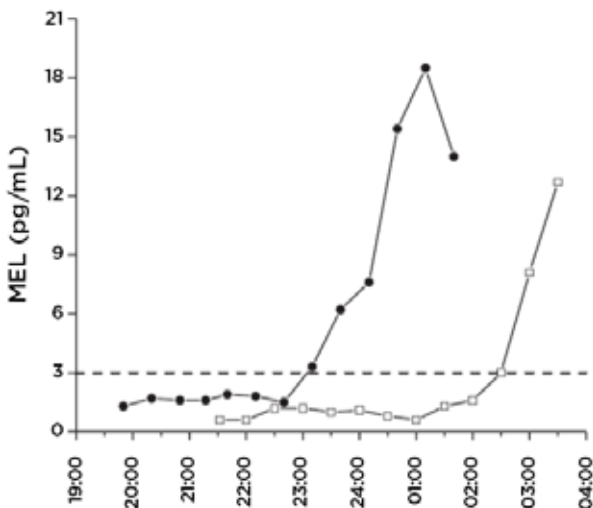


Figure 8: Examples of salivary melatonin levels, DLMO for a prototypical patient with delayed sleep phase syndrome (open squares) and a control subject (filled circles) (Wyatt et al., 2006).

PRODUCTS

30172091	Cortisol Saliva ELISA, RUO
RE62111	Cortisol Saliva LUM, IVD
RE52651	DHEA Saliva ELISA, RUO
RE52661	DHEA-S Saliva ELISA, RUO
30121045	17beta-Estradiol Saliva ELISA, IVD
RE62141	17beta-Estradiol Saliva LUM, IVD
30121046	Estriol high sensitive Saliva ELISA, RUO
RE52681	Estrone Saliva ELISA, RUO
DM59171	IgA Saliva ELISA, RUO
RE54041	Melatonin direct Saliva ELISA, RUO
RE52271	17-OH-Progesterone Saliva ELISA, RUO
RE52281	Progesterone Saliva ELISA, RUO
RE62021	Progesterone LUM, IVD
RE52631	Testosterone Saliva ELISA, RUO
RE62031	Testosterone LUM, IVD
RE80111	alpha Amylase Saliva Assay, RUO
RE69991.	/ SaliCap Set (collection tube), IVD
RE69995.	(Progesterone LUM only)

IVD: For in-vitro diagnostic use only

RUO: For research use only. Not for use in diagnostic procedures

LUM: Luminescence immunoassay.

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