
Testosterone

Physiology

In males the majority of testosterone is produced in the Leydig cells of the testicles. The release of testosterone is regulated by LH (luteinizing hormone) from the anterior part of the pituitary gland. The FSH (follicle stimulating hormone) from the same source has a positive effect on the Sertoli cells of the testicles which produce androgen binding protein and therefore activate development of the sperm cells mediated by the testosterone in the cells. Testosterone also suppresses the release of GNRH (gonadotropin releasing hormone) in the hypothalamic region and FSH and LH in the pituitary gland.

In females the testosterone level in blood is 10 to 20 times lower than in males. Half of the produced testosterone derives from the cortex of the adrenal glands and the other half from the ovaries. The release of testosterone from the ovaries is regulated via GNRH of the hypothalamic region by FSH and LH of the pituitary gland whereas the production of testosterone from the adrenal cortex is adjusted via the CRH (corticotropin releasing hormone) from the hypothalamic region by ACTH (adrenocorticotrope hormone) in the pituitary gland.

Normally the testosterone concentration in blood and saliva should be higher in the morning in females and males but there is no significant morning peak as with the diurnal cortisol profile. It has to be assumed that the testosterone level in men fluctuates in a manner similar to that of progesterone and estradiol in women because of the fluctuating release of GNRH and LH in the hypothalamic region and the pituitary gland.

In the blood circulation only 1 – 2 % of the testosterone is not bound to proteins. Only this free portion of testosterone has endocrine effects on the target cells. $\frac{1}{3}$ up to $\frac{4}{5}$ of the circulating testosterone is bound to SHBG (sex hormone binding globulin) the remaining part is fixed to albumin. As with other steroid hormones the levels of the binding globulins and therefore the concentration of bound testosterone is dependent on many physiological and pathological situations in humans. The fraction of free testosterone in blood can either be calculated by the values of the whole testosterone concentration and the SHBG concentration, which adds a level of variability since it is dependent on the result of two assays. Alternatively, free testosterone can be measured directly in serum. However, the quality of these assays is strongly dependent on the amount of the 50 to 100 times higher protein bound testosterone portion which is measured by the test, too. In the salivary glands the free testosterone is wholly separated from the protein bound fraction, and therefore the assessment of salivary testosterone levels is a reflection of the true level of active hormone.

Indications

Besides the main indications of testosterone measurement in endocrinology, i.e. diagnosis of hirsutism in women, evaluation of hormone dysregulation in children and differential diagnosis of hypoandrogenism in men, there are many other applications in psychology, sports medicine, anti-aging medicine and veterinary medicine for assessing the testosterone level in situations where blood sample collection is very difficult if not impossible (see also chapter cortisol: indications).

Some interesting clinical aspects of the assessment of salivary testosterone in publications are shown in the following paragraphs:

Gynaecology:

Comparison of the concentrations of various androgens in serum and the salivary testosterone level in healthy women and hirsutism patients points out that the latter analyte shows the best discrimination between the two groups.

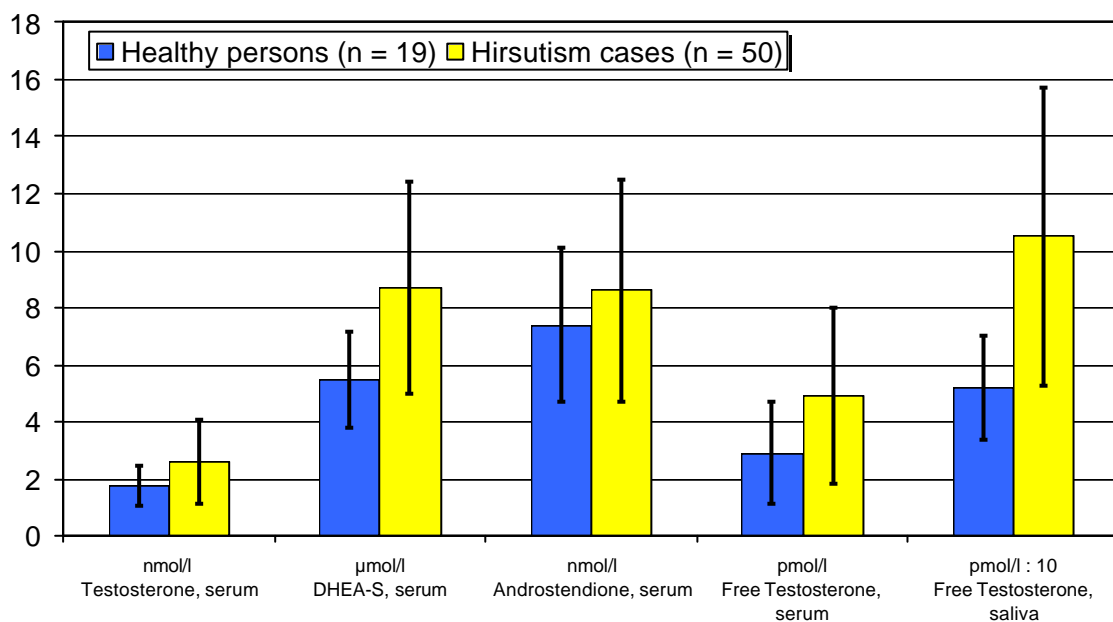


Fig. 38: Various androgen levels during hirsutism (J. Osredkar et al., 1989)

Variation of the testosterone level during the day in women is significant. Therefore taking multiple samples during the day has to be recommended. The success of antiandrogen therapy can also be evaluated by the measurement of the salivary testosterone level. There is a significant decrease in the testosterone concentration in the menstrual cycles of hirsute patients following therapy.

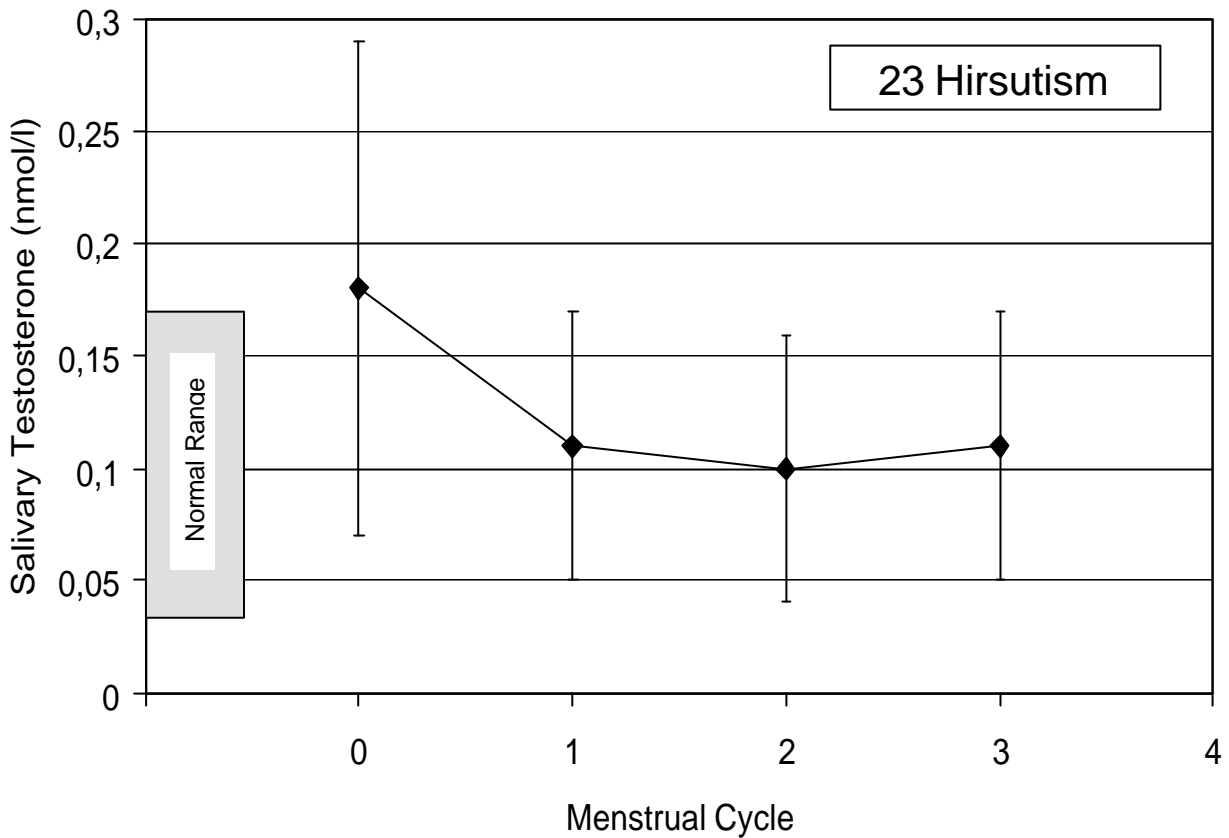


Fig. 39: Salivary testosterone level during antiandrogen therapy in hirsutism. Therapy: 100 mg cyproteronacetate daily from day 5 – 15; 50 µg ethinyl estradiol, 150 µg levonorgestrol daily from day 5 – 26 (J. M. Gomez et al., 1992).

Paediatrics:

Butler et al. (1989) described in a study with 84 boys (age 7.3 – 16.2 years) that the salivary testosterone concentration reflects accurately the gonadal function in this age group. They compared the mean daily salivary testosterone level of 6 samples with the clinical pupertal stages classified according to the TANNER schedule (G1 – G5; figure 40).

This result might be transferred to other age classes, so that there is a suggestion that even in older men the salivary testosterone level may reflect the gonadal function.

The following "values" may only be regarded as a guideline since they are dependent on variables such as the diagnostic assay used and population bias.

Age (years)	Female (pg/ml)*	Male (pg/ml)*
20 – 29	17 – 52	42 – 145
30 – 39	15 – 44	53 – 114
40 – 49	13 – 37	41 – 104
50 – 59	12 – 34	36 – 96
60 – 69	12 – 35	32 – 86
70 – 79	11 - 34	31 – 81
> 79		26 – 54
range at replacement therapy		30 – 60

* Levels taken in the morning

Test Characteristics

The IBL-Hamburg Testosterone in Saliva LIA has:

- a high **analytical sensitivity** (2.5 pg/ml)
- a good **specificity** (above all regarding 5a-Dihydrotestosterone)
- a good **precision** (functional sensitivity, see fig. below)
- a good **linearity** in the clinically relevant concentration range
- two levels of **saliva controls** included in the kit
- standards, controls and conjugate **ready for use**
- been applied on **automatized instruments**

The following table summarizes the test characteristics of the Free Testosterone in Saliva LIA of IBL-Hamburg (cat.no. RE 620 31)

FREE TESTOSTERONE IN SALIVA

- L I A -

Principle	Competitive Chemiluminescence Immunoassays		
Formate	12 microtiter strips with 8 wells each		
Sample	50 µl Saliva		
Standards	7 standards ready for use 0/6.4/16/40/100/240/760 pg/ml		
Incubation	4 hrs. (18 – 22 °C, plate shaker) 10 min. (18 – 22 °C, plate shaker)		
Substrate	Acridan based substrate		
Expected values	Lit. (RIA): Men:	9 – 12 a 13 – 60 a 60 – 80 a	10 – 70 pg/ml 30 – 140 pg/ml 10 – 50 pg/ml
	Women: premenopausal:		3 – 15 pg/ml
	Hirsutism:		7 – 30 pg/ml
	PCO Syndrome		14 – 45 pg/ml
Sensitivity	2.5 pg/ml		
Precision	Intra Assay:	4.0 – 8.1 %	at 30 – 110 pg/ml
	Inter Assay	7.4 – 11.7 %	at 30 – 110 pg/ml
Specifity	% cross reactivity (Abraham method)		
	5a-Dihydrostestosterone	1.6	
	Androstenedione	0.8	
	Methyltestosterone	0.4	
Controls	2 Saliva kit controls		
Automation	Assay is proved on different microtiterplate instruments		
Cat.-No.	RE 620 31		

Standard curves:

For the evaluation of the quality of a standard curve, the differentiation of the standard values in the low testosterone concentration range is important. The first concentration standard should have a signal value of below 90 % of that of the zero standard in a competitive immunoassay. Otherwise it is possible that under certain conditions, the first concentration standard might yield a higher value than the zero standard, resulting in an unacceptable standard curve and therefore a failed assay.

Secondly the 50 % intercept of a good standard curve should be near the middle of the concentration range defined by the standard values. If the 50 % intercept is shifted to the higher concentration values (i.e. in the standard curve of competitor 3) the differentiation of testosterone values in the low concentration range may be unacceptable.

Finally, a well designed standard curve for a competitive-binding immunoassay can be considered to be good quality if the highest concentration standard has a very low signal value, so that the whole range of signal differentiation is used by the curve.

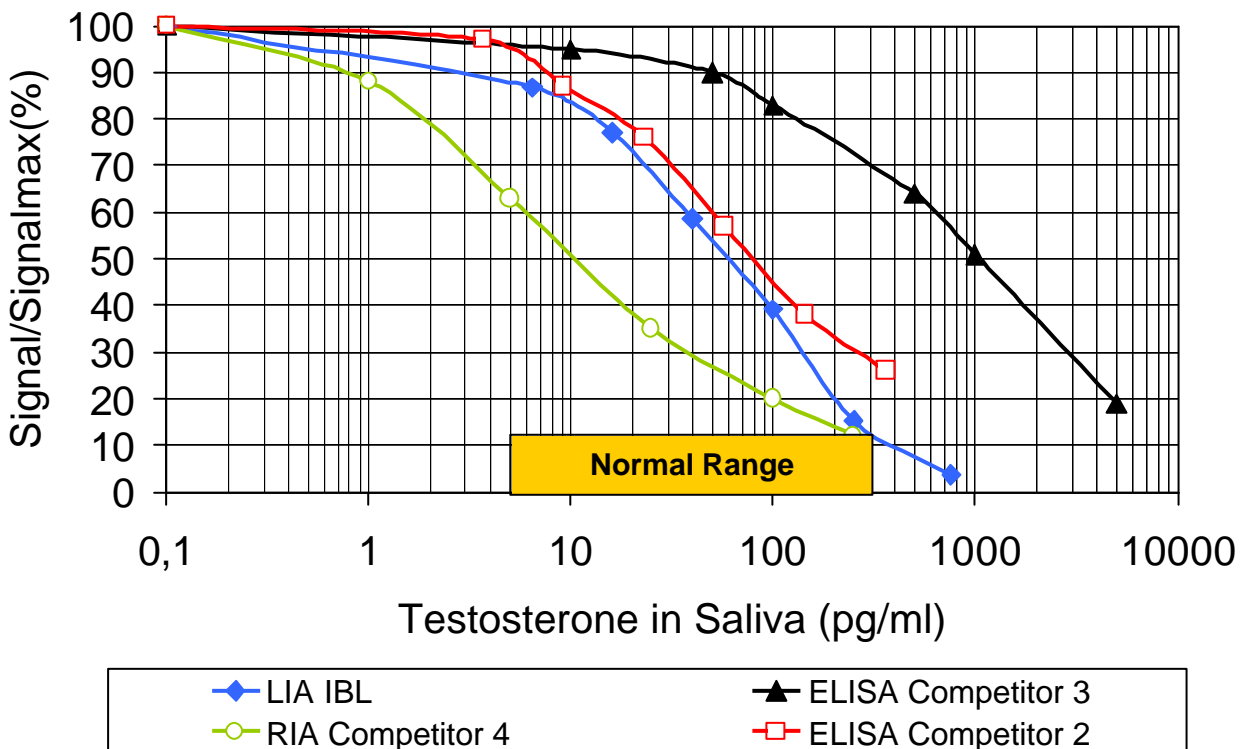


Fig. 41: Standard curves of free testosterone in saliva assays

Sensitivity:

In some Salivary Testosterone Assays a very good analytical sensitivity is claimed (< 1.5 pg/ml). It is important to know that analytical sensitivity is evaluated by replicate measurements of the zero standard, which consists of a buffer solution and not of saliva components!

More interesting is the reproducibility of the testosterone values of saliva samples (functional sensitivity). Coefficients of variation of replicate measurements below 10 % in the normal testosterone concentration range (i.e. 5 – 200 pg/ml) are acceptable. In figure 42 you can see that most salivary testosterone immunoassays don't fulfil this condition in the low concentration range.

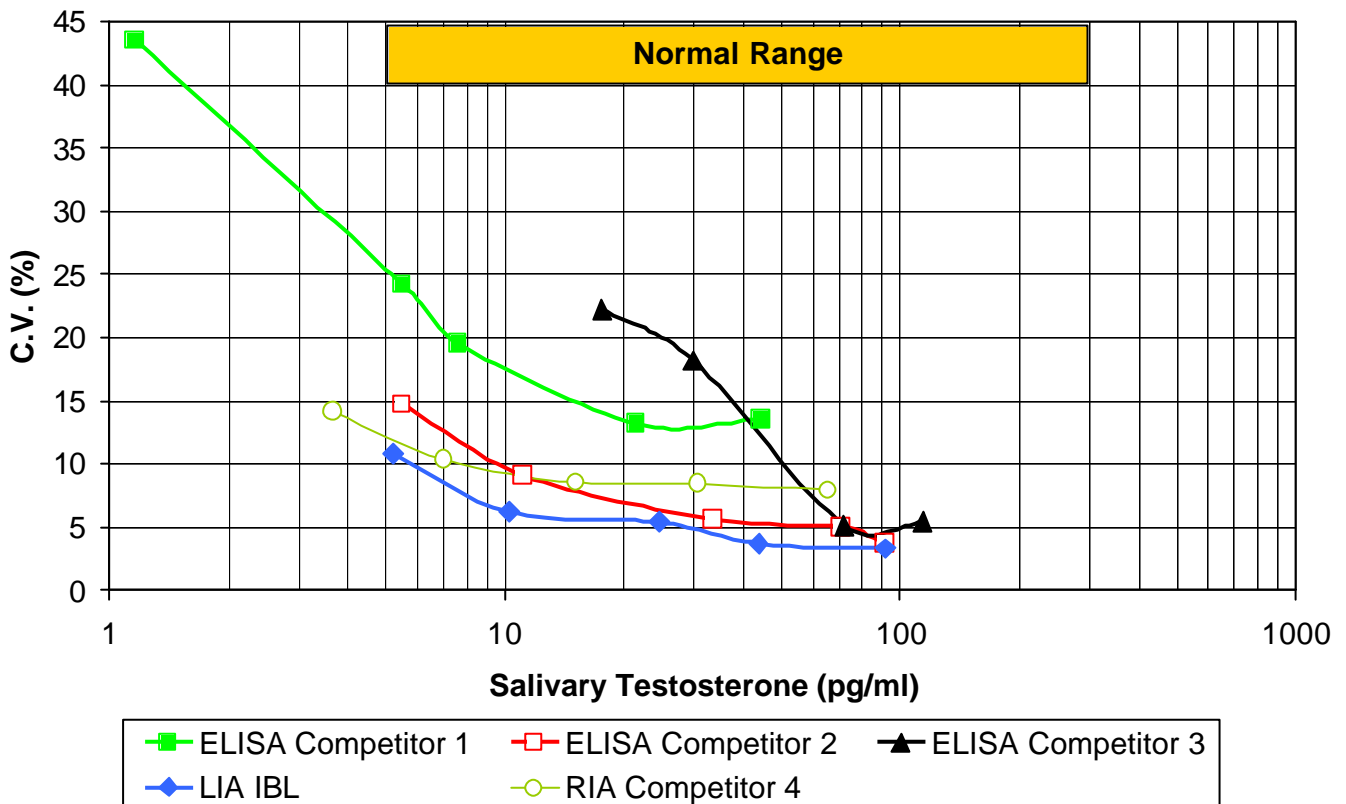


Fig. 42: Functional sensitivity of various Testosterone in Saliva Immunoassays
The testosterone level in eight replicates of several saliva samples was measured. In the figure the coefficients of variation are plotted against the testosterone concentration.

Literature

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